Water Resources Research Institute of the University of North Carolina Annual Technical Report FY 2016

Introduction

During 2016-2017 (Fiscal Year 2016), the Water Resources Research Institute (WRRI) of The University of North Carolina System was responsible for fostering and developing a research, training, and information dissemination program responsive to the water problems of the state and region. To develop its programs, the Institute maintains an aggressive effort to interact and communicate with federal, state, and local water managers and other relevant stakeholders. The close contact with these individuals is the tool used to ensure our research priorities stay at the forefront of an ever-changing landscape.

Research priorities continue to be identified and refined by the WRRI Advisory Committee, composed of 16 representatives of several federal and state agencies, local governments, industries, and non-governmental organizations (NGOs). Other water resource experts in the state with whom WRRI has close relationships are also consulted informally. A technical review committee is also convened on an annual basis to advise WRRI staff on the scientific merit of research proposals submitted for funding. Full-time faculty members from all North Carolina institutions of higher education are eligible to receive grants from WRRI.

In response to the student RFP jointly issued with NC Sea Grant in FY16, WRRI received a total of 28 student proposals from 6 institutions with a total funding request of $274,638. Five of these were selected for funding, totaling $49,966. Funds for these projects comes from USGS, state funds, and funds from two research consortia (the Urban Water Consortium and the Stormwater Group) administered by WRRI. Projects resulting from FY16 joint student RFP began in September 2016 and will conclude in August 2017. Results from those projects supported by USGS funds will be reported in the next USGS Annual Report.

WRRI did not issue a full faculty RFP in FY16 as the previous cycle had awarded several two-year projects, which pre-allocated the FY16 funding. Proposals that will be submitted to a current, open RFP will be ready for funding beginning in FY 18 and will be reported accordingly.

From RFP issued in FY15, 5 new research projects totaling $281,800 began during the FY16 reporting period. Of these, 3 were USGS-funded projects totaling $161,800. Two of the three are two-year projects and one is a one-year project, and progress and final results are reported in the following sections. The remaining projects were supported by state funds, the Urban Water Consortium and the Stormwater Group (for more information about WRRI's activities with these two groups, please see the progress report for the Information Transfer Program).

WRRI funding was used to support a total of fifteen PhD students, six master's students and twelve undergraduate students. Nine faculty were supported through Institute-funded projects during this cycle. An additional 65 students participated in the WRRI annual conference.

The information transfer program continued to focus on disseminating results of sponsored research and providing information on emerging water issues, solutions, and regulations. Results of research are disseminated by publication of technical completion reports, peer reviewed manuscripts, summary posts on the WRRI website, and presentations by investigators at the WRRI Annual Conference and individual group meetings. Five peer-reviewed publications from WRRI projects were published during this period.

Through the WRRI Center for Watershed Excellence (CEWM), the NC Watershed Stewardship Network (WSN) continued its engagement of watershed professionals and volunteers across the state. This year, the WSN hosted a "Tools of Watershed Management" workshop series that engaged 42 watershed stewards in 3 different workshops across the state to learn tools for watershed planning, restoration and protection. Additional watershed protection activities managed by WRRI's Sustainable Waters and Communities Coordinator are highlighted in the Notable Awards and Achievements section and in the Information Transfer

progress report.

WRRI continues to be a sponsor of continuing education credits by the NC Board of Examiners of Engineers and Surveyors and the NC Board of Landscape Architects. This allows WRRI to offer Professional Development Hours (PDHs) and contact hours for attendance at the WRRI Annual Conference and other workshops and seminars that WRRI sponsors. This year, WRRI formed a new working relationship with industry partner Duke Energy, and held a day-long workshop that provided 6.5 PDHs to 170 in-person attendees and several other groups around the state who participated virtually via webinar.

WRRI continues to maximize staff efficiencies and outputs. The program leverages funds from a variety of sources such as the Urban Water Consortium, the Stormwater Group, and grants received by the Center of Excellence for Watershed Management. WRRI team members are actively engaged in board and committee activities around the state where they bring expertise and perspective to efforts to address NC's water issues. These additional inputs help WRRI to expand the reach and impact of research and outreach activities, and grow involvement in and support of water-related research and outreach across the state.

Research Program Introduction

During 2016-2017 (Fiscal Year 2016), WRRI continued its focus of fostering research, training, and information transfer that is responsive to water issues of the state and region. Results from Institute-supported research efforts are expected to assist local, municipal, state, regional and federal agencies to improve their decision-making in the management and stewardship of their water resources. WRRI continued to expand its engagement of students through another graduate student request for proposals (RFP) and more targeted tracking of student activities.

To help it chart and sponsor a research program responsive to the water resource issues and opportunities in North Carolina, WRRI interacts closely with state agencies such as the NC Department of Environmental Quality, water and power utilities, and an array of research and outreach programs within the UNC system and at private higher educational institutions across North Carolina. The Institute's advisory committee provides input, guidance, and review of the research priorities that are used in developing our Requests for Proposals (RFPs) and directing other research activities. This committee is composed 16 representatives of several federal and state agencies, local governments, industries, and non-governmental organizations (NGOs). In early 2017, the committee convened in person in Raleigh for a thorough discussion of the state's most pressing water issues and how WRRI's research priorities and programs could address these issues.

Based on in-depth discussions with stakeholders and advisory committee members regarding the most significant water research needs and priorities in NC, WRRI's research priorities are captured within four main RFP focus areas. Research priorities are incorporated into our Section 104b Objectives on an annual basis and included in our RFP. The RFP is sent to relevant offices of sponsored research at colleges and universities as well as an email distribution list of approximately 180 university faculty across North Carolina. Full-time faculty members from all North Carolina institutions of higher education are eligible to receive grants from WRRI. However, during this reporting cycle, WRRI did not issue a solicitation for faculty research proposals as FY16 funds were pre-committed to several two-year projects from the previous RFP. WRRI did issue a graduate student RFP in conjunction with NC Sea Grant.

In response to the student RFP jointly issued with NC Sea Grant in FY16, WRRI received a total of 28 student proposals from 6 institutions with a total funding request of $274,638. Five of these were selected for funding, totaling $49,966. Funds for these projects comes from USGS, state funds, NC Sea Grant and funds from two research consortia (the Urban Water Consortium and the Stormwater Group) administered by WRRI. Projects resulting from FY16 joint student RFP began in September 2016 and will conclude in August 2017. Results from those projects supported by USGS funds will be reported in the next USGS Annual Report.

From RFP issued in FY15, 5 new research projects totaling $281,800 began during the FY16 reporting period. Of these, 3 were USGS-funded projects totaling $161,800. Two of the three are two-year projects and one is a one-year project, and progress and final results are reported in the following sections. The remaining projects were supported by state funds, the Urban Water Consortium and the Stormwater Group (for more information about WRRI's activities with these two groups, please see the progress report for the Information Transfer Program).

Role of organic nitrogen to eutrophication dynamics along the Neuse River Estuary, NC

Basic Information

Title:	Role of organic nitrogen to eutrophication dynamics along the Neuse River Estuary, NC
Project Number:	2016NC198B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NC-03
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Focus Category:	Nutrients, None, None
Descriptors:	None
Principal Investigators:	Hans Paerl, Alexandria Graves Hounshell

Publications

There are no publications.

ROLE OF ORGANIC NITROGEN TO EUTROPHICATION DYNAMICS ALONG THE NEUSE RIVER ESTUARY, NC

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Abstract

Role of organic nitrogen to eutrophication dynamics along the Neuse River Estuary, NC

The Neuse River Estuary (NRE), North Carolina has been experiencing symptoms of eutrophication (as harmful algal blooms, hypoxia/anoxia, and fish kills) since the early 1980's. Despite reductions in phosphorus loading during the late 1980's, negative impacts of eutrophication persisted and in the mid-1990's a total maximum daily load was enacted to reduce nitrogen loading to the estuary. Since the 1990's there has been a documented decrease in inorganic nitrogen loading but a proportional increase in organic nitrogen loading to the system. During the same time period there has been increasing urban and agricultural development in the watershed leading to changes in nitrogen source loading to the NRE. To capture the changing nitrogen sources, specifically as organic nitrogen sources, a year-long environmental survey was conducted. Dissolved organic matter and particulate organic matter samples were collected, in conjunction with UNC-CH ModMon sampling program, from July 2015 – July 2016. Both dissolved and particulate organic matter samples were collected and analyzed for fluorescence spectroscopy using Excitation Emission Matrices (EEMs). EEMs coupled with the statistical decomposition technique, Parallel Factor Analysis (PARAFAC) can allow for identification and tracking of broad organic matter classes. By correlating the identified organic matter components with auxiliary biogeochemical data collected by the ModMon program, it was possible to determine the bio-reactivity of specific organic matter classes. While the organic matter pool was largely dominated by natural, terrestrial organic matter classes, results suggest there are bio-reactive fractions of the organic matter pool (as proteins and microbially produced components), produced both in the watershed and in-situ, which may stimulate primary production and lead to negative impacts (algal blooms, hypoxia/anoxia, and fish kills) associated with eutrophication. Additional research is needed to pinpoint the exact source of these biologically active components in the watershed and those that are autochthonously produced.

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Acknowledgements

We appreciate the technical assistance with water collection, sample processing, and sample analysis provided by Betsy Abare, Jeremy Braddy, Lois Kelly, Karen Rossignol, and Randy Sloup. Chris Osburn and Ben Peierls provided thoughtful and useful discussions and feedback. Additional funding was provided by NC DENR Division of Water Quality and the Lower Neuse Basin Association/Neuse River Compliance Association in support of the ModMon environmental survey program which provided background data and boat support.

1. Introduction

Nutrient over-enrichment is a broadly recognized problem in estuarine and aquatic ecosystems. Harmful effects include eutrophication, harmful algal blooms, hypoxia/anoxia, and fish kills, which negatively impact the resources (fisheries, recreation, tourism, drinking and irrigation water source) of impacted systems. In many estuarine ecosystems, including North Carolina's Neuse River Estuary (NRE), nitrogen (N) is the principal limiting nutrient for primary production (Rudek et al., 1991; Paerl, 2009). Increased N loading to the NRE due to accelerated urbanization, and agricultural and industrial activities in the watershed have led to increased algal biomass, including harmful algal blooms, and habitat degradation (Paerl, 2009). In ecosystems that exhibit accelerating eutrophication, including the NRE, total maximum daily loads (TMDLs) have been developed to reduce N loading (Paerl et al., 2004). While progress has been made in reducing inorganic N loading, a parallel increase in organic N (ON) has been reported in watersheds undergoing human development (Pellerin et al., 2006), including in the NRE, where Lebo et al., 2012 documented a decrease in inorganic N loading since the introduction of the TMDL in 1999, but a proportional increase in ON loading.

Little is known about the sources, fates, and bio-reactivity of ON through the freshwater-estuarine continuum. Laboratory and experimental evidence suggests anthropogenic ON sources from urban runoff, agriculture, and wastewater treatment effluent will promote algal and bacterial growth in aquatic ecosystems (Seitzinger et al. 2002; Berman and Bronk 2003; Bronk et al 2010). Little is known, however, about whether natural phytoplankton and bacterial assemblages use DON or PON sources in-situ. The study addressed two main questions:

> **Question 1:** What is the extent of transport and the fate of natural and anthropogenic particulate ON (PON) and dissolved ON (DON) sources in the N-sensitive NRE?
>
> **Question 2:** What is the bioreactivity of ON along the freshwater-estuarine continuum? Are ON signatures changing in magnitude (i.e., concentration) through the NRE, indicating degradation or utilization by phytoplankton and microbial communities?

In order to address these questions, a year-long environmental survey was conducted in the NRE in coordination with the existing UNC-CH sampling program, ModMon (http://www.unc.edu/ims/neuse/modmon/) (Paerl et al., 2014). DON and PON samples were collected and analyzed using excitation-emission matrix (EEM) fluorescence spectroscopy coupled with the statistical decomposition technique parallel factor analysis (PARAFAC) to partition and model sources of ON to the NRE by their discrete fluorescence signatures (Brym et al., 2014; Osburn et al., 2012; Osburn et al., 2016). The EEM-PARAFAC technique relies on the absorbance and fluorescent patterns of different, broad OM classes that exist in natural aquatic systems. Classes of organic matter contained within EEMs were first identified and classified by Coble, 1996. Much of the classification and naming of these fluorescence peaks has persisted in the literature (Figure 1; Table 1) (Coble et al., 2014; Osburn et al., 2012).

Table 1. Peak designation, excitation maxima, emission maxima, and the associated organic matter class for the most commonly identified EEM-PARAFAC fluorescence peaks (Coble, 1996; Coble et al., 2014; Osburn et al., 2012).

Peak Designation	Excitation Maximum (nm)	Emission Maximum (nm)	Organic matter class
A	< 250	400-460	Terrestrial, humic-like; fulvic acid
C	320-360	420-460	Terrestrial, humic-like, allochthonous
M	290-310	370-410	Autochthonous, microbial humic-like
T	275-280	340-344	Autochthonous, protein, tryptophan

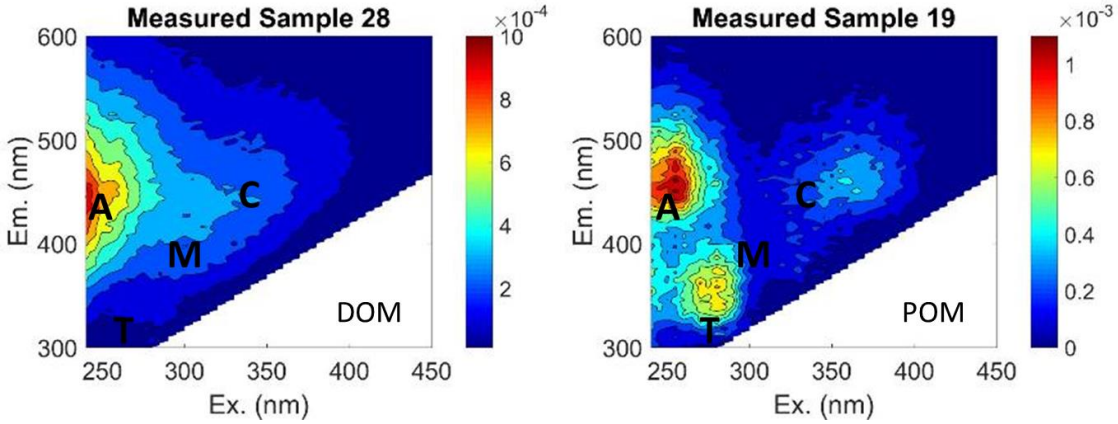

Figure 1. Representative DOM and POM EEMs collected for samples from the NRE. Locations of the commonly identified fluorescence peaks as originally described by Coble, 1996 are indicated by the letters and correspond to the peak designation listed in table 1.

Representative DOM and POM EEMs collected from the NRE are plotted in Figure 1. The DOM sample is representative of the terrestrial influence common in the DOM pool for samples in the NRE and includes the characteristic broad, single peak of fluorescence in the mid-emission wavelengths. The POM sample exhibits the common 'three-peak pattern' observed in estuarine and marine POM samples (Brym et al., 2014). The three-peak pattern is a combination of fluorescence from protein (tryptophan, 'T') and a component that has been correlated with recent microbial activity (Brym et al., 2014). Using EEMs coupled with PARAFAC, broad organic matter classes can be identified and tracked through aquatic ecosystems and the sources and bioreactvity of these classes can be assessed.

2. Methods

2.1 Environmental Surveys

A year-long environmental survey was conducted in the NRE from July 20, 2015 to July 18, 2016 in coordination with the existing UNC-CH ModMon sampling program (Paerl et al., 2014). 11 stations were sampled along the main axis of the estuary from the head near Streets Ferry Bridge to the lower estuary near the Pamlico Sound either monthly (November – February) or twice monthly (March – November) (Figure 2). Samples were collected from both surface (0.5 m below surface) and bottom (~0.5 m from bottom) at all stations.

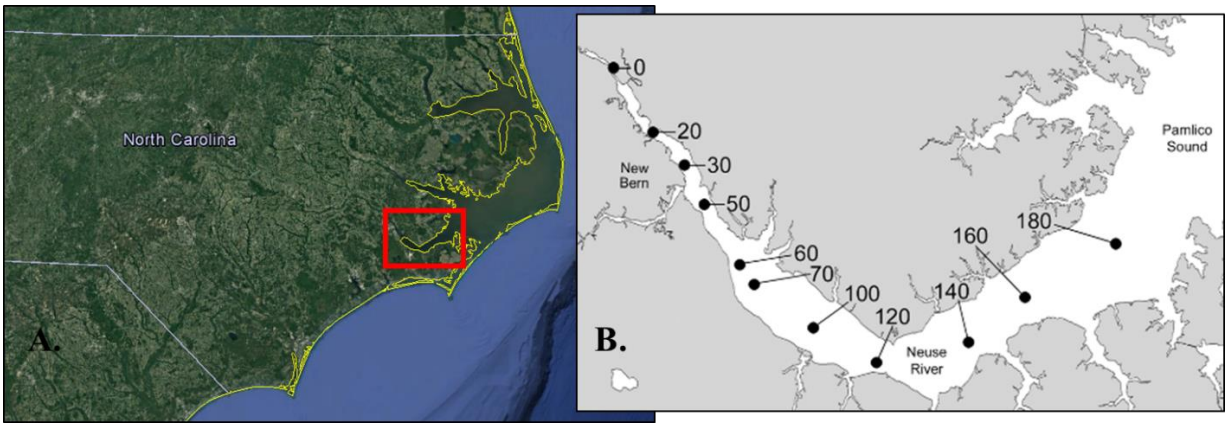

Figure 2. A. Location of the NRE within eastern North Carolina. B. ModMon sampling locations in the NRE from Streets Ferry Bridge (Station 0) to the Pamlico Sound (Station 180). Figure adapted from Paerl et al., 2014.

For DON and PON analysis, about 100 mL of collected NRE sample was filtered through a combusted 0.7 µm GF/F filter. The filter was collected for PON analysis via the base extracted particulate organic matter (BEPOM) method (Brym et al., 2014) and the filtrate collected for DOM analysis (Osburn et al., 2012). Both BEPOM and DOM samples were analyzed for fluorescence spectra as excitation emission matrices (EEMs) on a Cary Varian Eclipse Spectrophotometer. Excitation wavelengths were measured from 240 to 450 nm every 5 nm and emission wavelengths were measured from 200 to 600 nm at 2 nm intervals. Prior to analysis, both DOM and POM samples where filtered through a 0.2 µm filter. Instrument excitation and emission corrections were applied to each sample EEM as well as corrections for inner-filtering effects, calibration against the Raman signal of Nanopure water or sodium hydroxide for DOM and BEPOM analysis respectively, and standardized to quinine sulfate units (QSU) (Osburn et al., 2012; Stedmon & Bro, 2008). Absorbance scans used for EEM correction were analyzed on a Shimadzu UV-1700 PharmaSpec measured from 200 nm to 800 nm.

In addition to samples collected for DON and PON analysis, a range of other biogeochemical parameters were collected and analyzed by the ModMon sampling program (i.e., temperature, salinity, $NO_{2/3}^-$, NH_4^+, TDN, DON, PO_4^{3-}, chlorophyll-*a*). Neuse River discharge measurements were obtained from the USGS gage #02091814 near Fort Barnwell, NC located about 26 km upstream from New Bern (Paerl et al., 2014).

2.2 PARAFAC Modeling

The statistical decomposition technique Parallel Factor Analysis (PARAFAC) is applied to a set of collected DOM and/or POM EEM samples to mathematically identify and separate broad classes of organic matter inherent to the samples (Stedmon and Bro, 2008). By using the coupled EEM-PARAFAC technique, broad organic matter classes can be identified and tracked through aquatic systems and the transport, fate, and bioreactivity of organic matter can be assessed (Fellman et al., 2011; Jaffé et al., 2014; Markager et al., 2011). Three PARAFAC models were generated for the DOM (n = 471) and POM (n = 163) samples collected from the NRE: a DOM model that only contained DOM samples, a POM model that only contained POM samples, and a combined POM+DOM model that contained both POM and DOM samples. Each PARAFAC model was developed using the DOMFluor Toolbox in Matlab (Stedmon and Bro, 2008). All EEMs were normalized to their maximum fluorescence prior to PARAFAC modeling (Osburn et al., 2012).

2.3 Application of FluorMod

In addition to developing new PARAFAC models fit to the DOM and POM samples collected during the described sampling, a previously developed mixing model and PARAFAC model, FluorMod was fit to the collected NRE samples (Osburn et al., 2016). FluorMod is a mixing model based on PARAFAC components identified from samples collected in the upper Neuse River watershed (Figure 3). PARAFAC components identified in the watershed samples contained both humic-like, terrestrial DOM signatures and potentially biologically reactive DOM signatures that are indicative of recent biological activity (C3, protein – tryptophan; C5, protein – tyrosine; and C8 – microbial activity).

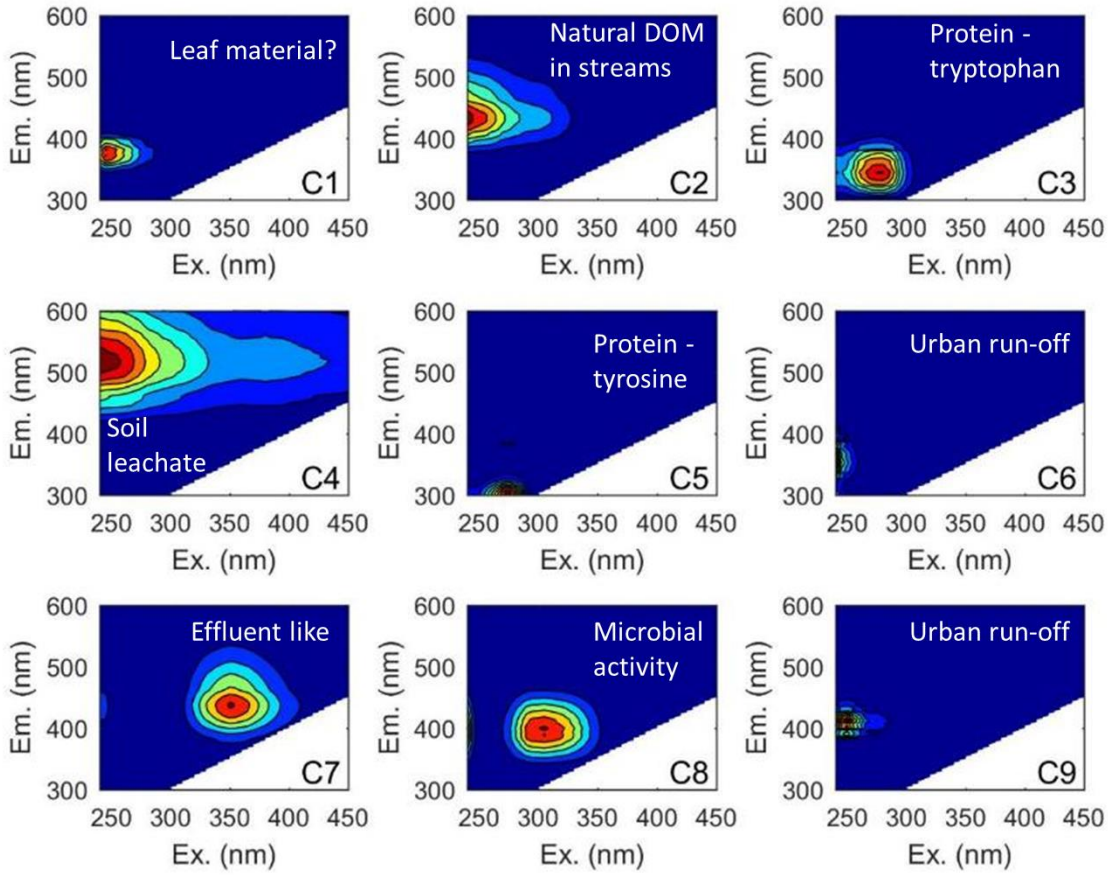

Figure 3. FluorMod PARAFAC model components identified by designations assigned by Osburn et al., 2016. (Reproduced from Osburn et al., 2016).

To develop the FluorMod additive mixing model, samples of DON sources collected in the watershed were characterized and assigned differing proportions of the 9 identified PARAFAC components. 8 DON sources from the Neuse River watershed were characterized: reference, wastewater treatment facility (WWTF) effluent, WWTF influent, poultry leachate, swine lagoon, septic outflow, street runoff, and soil leachate (Osburn et al., 2016). The additive mixing model, FluorMod, can be applied to samples collected from the Neuse watershed, river, or estuary to determine the relative proportion of each identified watershed source within the water sample. The goal of FluorMod is to identify and track watershed sources of DOM through the Neuse River and NRE. The application of FluorMod both as a mixing model and as a PARAFAC model to estuarine samples was assessed during this study.

2.4 Statistics

All PARAFAC modeling was conducted in Matlab version 2016a using the DOMFluor toolbox (Stedmon and Bro, 2008). PARAFAC model comparisons were conducted using TuckMatch in Matlab to determine if components identified in the three PARAFAC models (DOM, POM, and POM+DOM) matched with > 95% similarity (Lorenzo-Seva & Berge, 2006). Correlations were tested using the Spearman rank correlation. Principal components analysis (PCA) was conducted using princomp in Matlab. Prior to conducting PCA all measurements were normalized to their z-score.

3. Results

3.1 Environmental Surveys

The time period from July 2015 to July 2016 was relatively wet with discharge higher than average for the period from November 2015 – March 2016 (Figure 4, USGS). Increased discharge has a number of impacts on the estuary and can include decreased salinity, elevated nutrient loading, and movement of the chlorophyll-a maximum further downstream (Paerl et al., 2014). It is important to note, that because the USGS gaging stations is upstream from the head of the estuary, impacts of these freshwater pulses to the estuary may not be observed at monitored stations until a few days to weeks after the discharge increases at the gaging station.

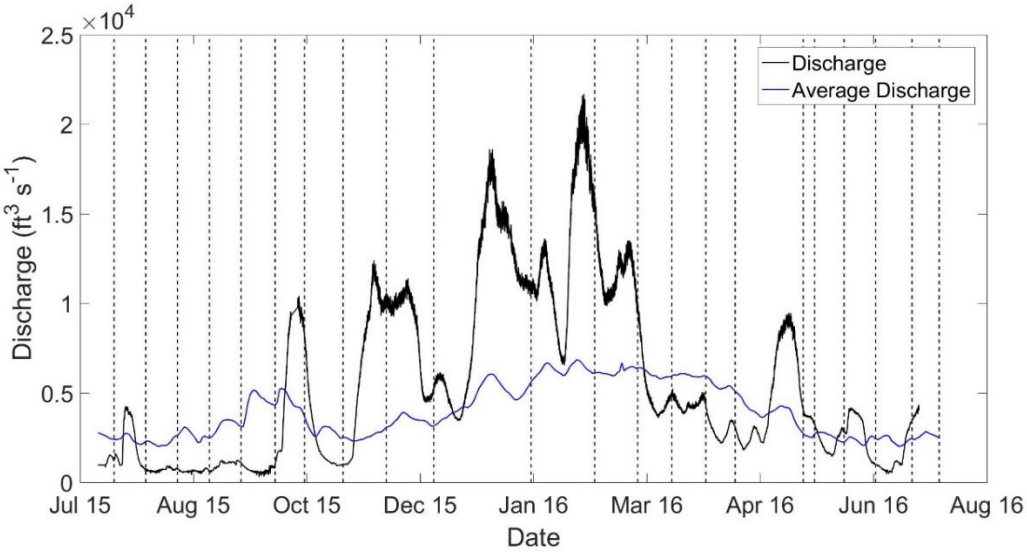

Figure 4. Discharge for the Neuse River measured at Ft. Barnwell, NC from July 13, 2015 to July 8, 2016. There was no discharge data between July 8, 2016 and July 18, 2016. Dates of sampling campaigns are indicated by the vertical dashed lines (total of 22 samplings). The solid black line is the measured discharge for the time period; the solid blue line is the average discharge calculated from historic data for each calendar day.

One way to assess the impact of freshwater discharge events on the estuary is by salinity. Salinity in the upper estuary (Station 0) remained low (<2 PSU) for the entire sampling period (Figure 5), indicating this station was essentially a river-dominated station throughout the study. Salinity at station 180 was also influenced by the wet winter. Lower salinity, freshwater pulses are often associated with higher concentrations of nutrients and organic matter which, based on salinity measurements, could have an impact throughout the estuary from station 0 to 180 (Paerl et al., 2014).

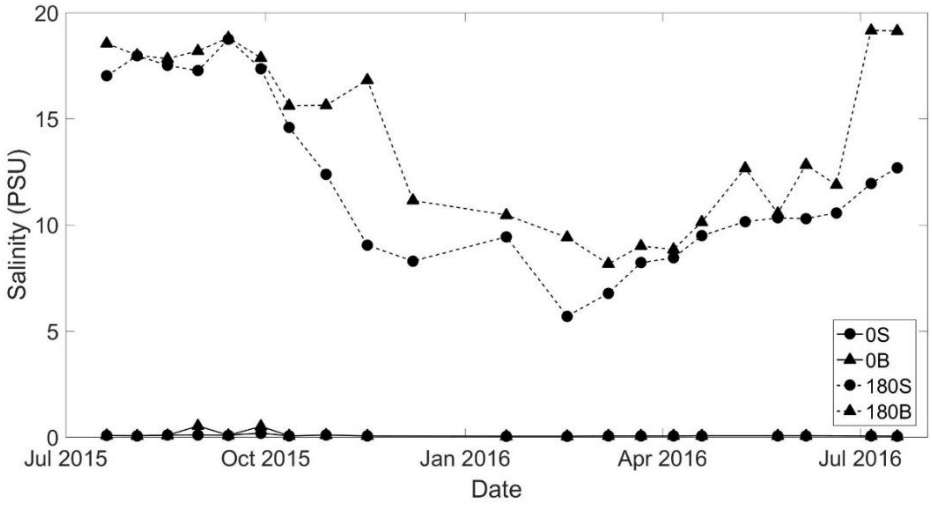

Figure 5. Salinity plotted for the head (station 0) and most downstream location of the estuary (station 180) for both surface and bottom.

In addition to salinity, nitrate concentrations were also affected by the freshwater pulses (Figure 6). Nitrate concentrations at station 180 were below detection for essentially all time points, except for two time points in the spring of 2016 when salinity was lowest. Station 0 had relatively high nitrate concentrations for the sampling period with increases in concentration during periods of high freshwater discharge.

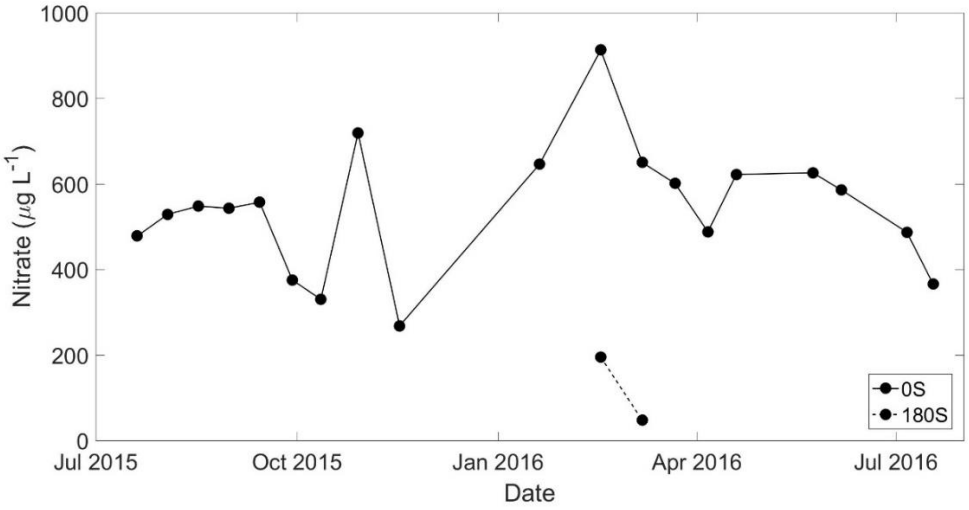

Figure 6. Nitrate plotted for surface samples collected at station 0 and station 180. Nitrate concentrations at station 180 were below detection except for two points in the spring of 2016.

DON concentrations were also measured for surface samples at station 0 and 180 (Figure 7). As with nitrate, DON concentrations are generally lower at the mouth of the estuary (station 180) compared to the upper estuary (station 0). Unlike nitrate concentrations, DON doesn't appear to be coupled with freshwater discharge and decreased at both stations when discharge was highest during spring of 2016.

Additionally, the concentration difference between station 0 and 180 is much smaller for DON concentrations compared to nitrate concentrations.

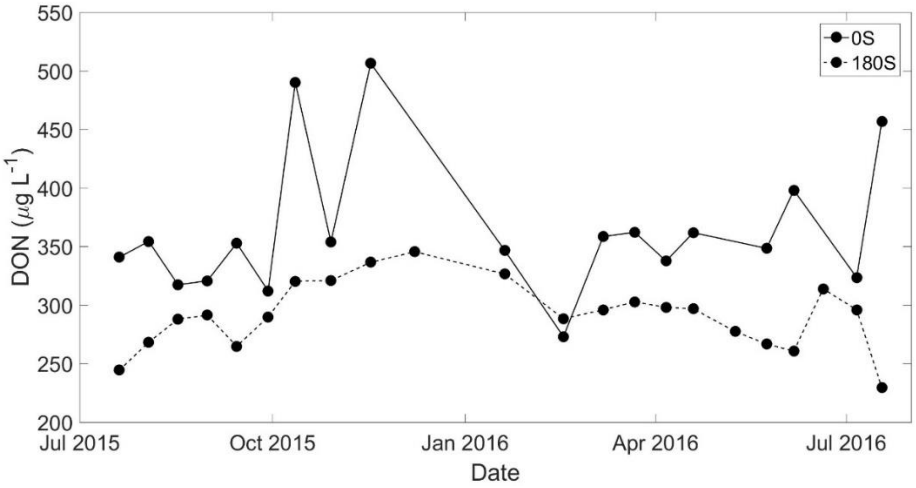

Figure 7. DON plotted for surface samples collected at station 0 and station 180.

Chlorophyll-*a* is plotted for surface samples at station 0 and 180 (Figure 8). Phytoplankton biomass is low in the upper estuary, however, in the lower estuary chlorophyll-*a* increases with freshwater discharge and nitrate concentration. Elevated chlorophyll-*a* levels are observed throughout winter 2015 and spring 2016. There are several instances where the chlorophyll-*a* concentrations at station 180 exceeded the state mandated threshold of 40 µg L^{-1}.

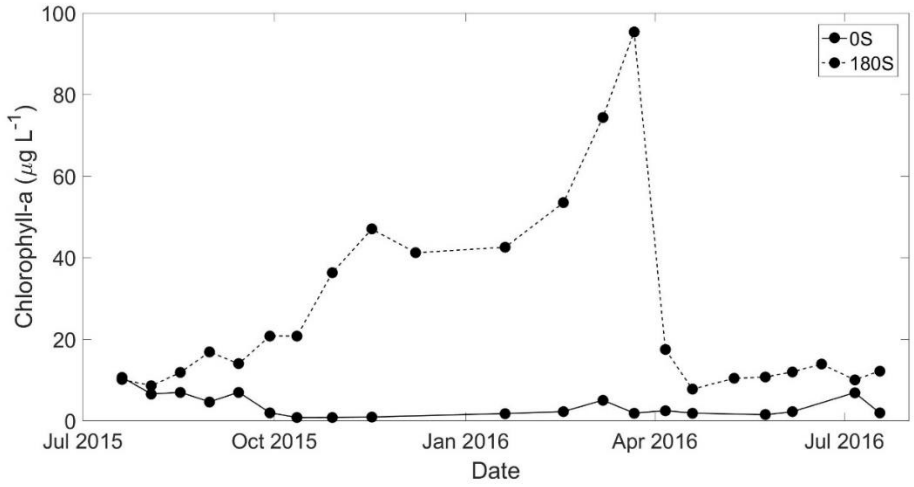

Figure 8. Chlorophyll-*a* plotted for surface samples collected at station 0 and station 180.

3.2 PARAFAC Modeling

Three PARAFAC models were developed based on samples collected in the NRE: 1. a DOM model fitted to only DOM samples, b. a POM model fitted to only POM samples, and c. a POM+DOM model fitted to both POM and DOM samples. All three models were split-half validated. Residual models were generated for each of the three original PARAFAC models. Residual models apply a new PARAFAC model to residuals calculated from the raw EEM and the EEM modeled with the original PARAFAC model. By

analyzing model residuals in this way, it is possible to assess the robustness of a model and it's capability to accurately capture all fluorescence signals in the samples.

Mathematically distinct components as identified by PARAFAC, are then compared to previously conducted studies that have linked these statistically distinct PARAFAC components with their chemical composition, in an online database, OpenFluor (Murphy et al., 2014). In this way, broad classes of organic matter can be identified from each sample and tracked through a given system.

a. DOM PARAFAC Model

A three component PARAFAC model was fitted to DOM samples collected from the NRE (n = 471). Two of the components identified are considered terrestrial, humic-like while component 2 is thought to be a humic-like, microbial component and an indicator for recent biological production (Figure 9; Table 2).

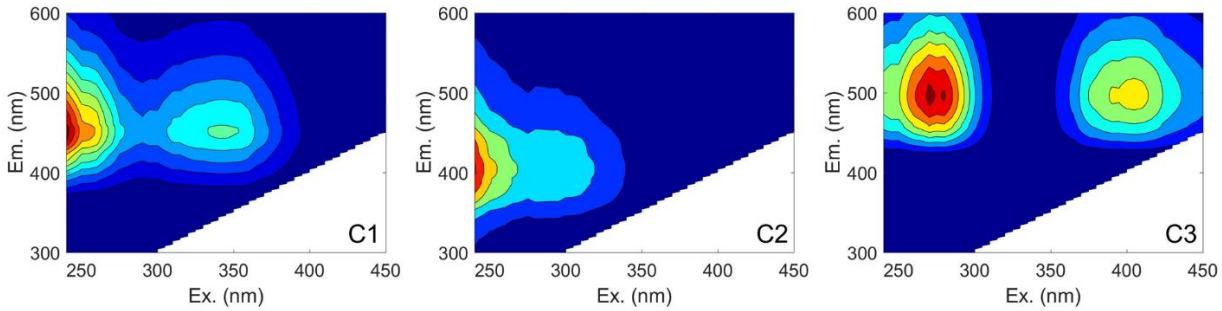

Figure 9. Components as identified by PARAFAC modeling of DOM samples (n = 471).

Table 2. Excitation maximum, emission maximum, number of matches to OpenFluor, and assignment corresponding to previous studies for the 3-component PARAFAC DOM model.

DOM Component	λ_{ex} (nm)	λ_{em} (nm)	Matches to OpenFluor	Assignment corresponding to previous studies
C1	<240, 340	452	14	Humic, fulvic-acid like; terrestrially derived; combination of A and C peaks (Osburn et al., 2016)
C2	<240	406	6	Microbial, humic-like; potentially from phytoplankton exudates; eutrophic estuaries; similar to M-peak (Yamashita et al., 2013)
C3	270, 205	496	4	Humic-like; terrestrially derived (Cawley et al., 2012; Yamashita et al., 2013)

A six component model was generated based on DOM sample residuals (Figure 10; Table 3). The model was not split-half validated nor did any of the components match in OpenFluor. Despite this, the residual model appears to be capturing components that do reflect organic matter fluorophores. Components were assigned to organic matter classes using data from past literature not included in OpenFluor.

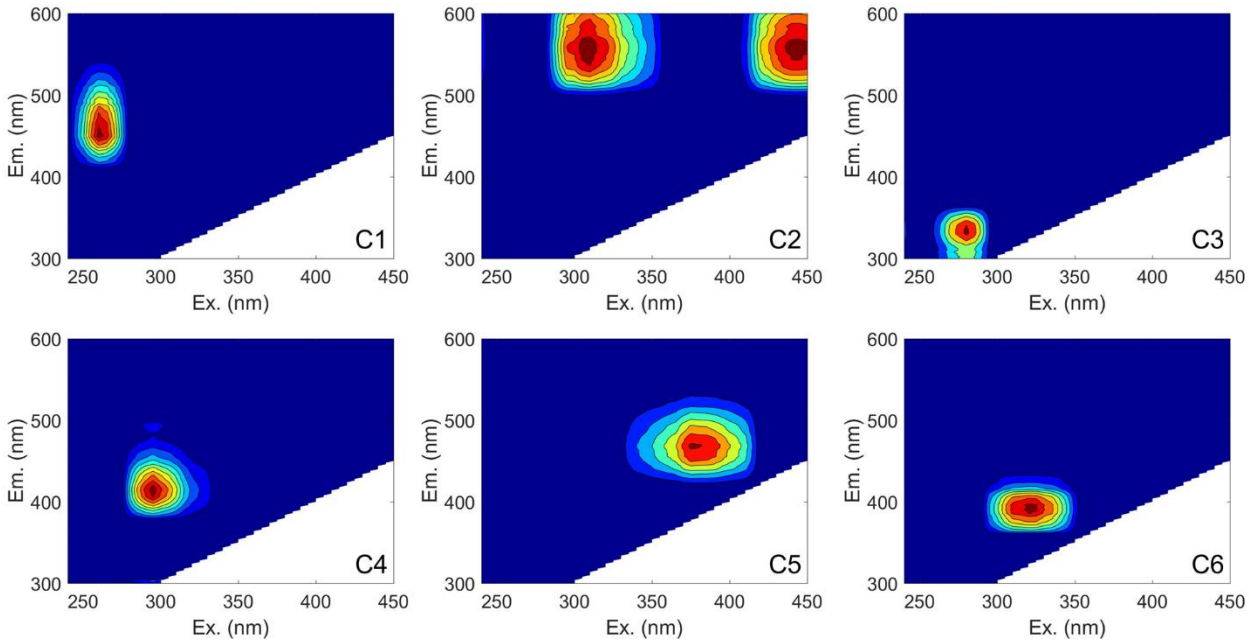

Figure 10. 6 component PARAFAC model identified based on DOM sample residuals. The model was not split half validated.

Table 3. Excitation maxima, emission maxima, and possible component assignment for the 6 components identified in the DOM residual PARAFAC model. None of the components matched in OpenFluor. Designations were assigned based on studies conducted prior to the development of the OpenFluor database.

DOM Component	λ_{ex} (nm)	λ_{em} (nm)	Possible component assignment
C1	260	450	Peak A; UVC Humic-like (Coble, 2007); aromatic, derived from terrestrial plant material (Fellman et al., 2010)
C2	310, 445	558	Terrestrial, humic-like, aromatic (Fellman et al., 2010)
C3	280	332	Protein signal, possibly tryptophan (Coble, 2007)
C4	305	414	Ferulic acid (Wünsch et al., 2015); Microbial (Coble, 2007)
C5	375	470	Soil fulvic acid, from freshwater systems; Schiff-base derivative (Senesi, 1990)
C6	320	390	In the general region of microbial activity, un-characterized

The residual PARAFAC model appears to capture more biologically produced, autochthonous sources of DOM than the original fitted DOM PARAFAC model. The original DOM samples were overwhelmingly dominated by terrestrially-derived allochthonous sources of DOM. When these sources were modeled and essentially removed during the original PARAFAC model, it was then possible to capture and model the more autochthonous components in the residual PARAFAC model.

It is hypothesized that many of the components captured by the residual PARAFAC model are 'metastable', meaning these organic matter molecules exist in the estuary as an intermediate phase as DOM is degraded (via photochemical or biological processes). These states are hypothesized to be fleeting and capturing them in any given PARAFAC model is difficult as, often the concentrations of these states are low and time scales too short to detect (Stedmon & Cory, 2014). Previous studies have

also determined the biologically active, labile DOM pool is quickly cycled, meaning while these components are constantly produced in-situ, they are also constantly consumed such that the concentration of these molecules at any given time point is low (Repeta, 2015; Sipler and Bronk, 2015). This makes capturing these biologically active components in the DOM pool difficult.

b. POM PARAFAC Model

A five component PARAFAC model was fitted to surface and bottom POM samples (n = 163) (Figure 11; Table 4). The model is a mixture of both terrestrial and autochthonous sources of OM fluorescent components and captures the characteristic 'three-peak' POM fluorescence signature (Brym et al., 2014). The POM model appears to capture more fluorescence variability than the DOM PARAFAC model. The identified components are similar to fluorescence components identified for a POM+DOM PARAFAC model developed on samples collected from nutrient addition bioassays conducted in the NRE system (Hounshell et al., unpublished). The model was split-half validated and identified components matched with previous models on OpenFluor.

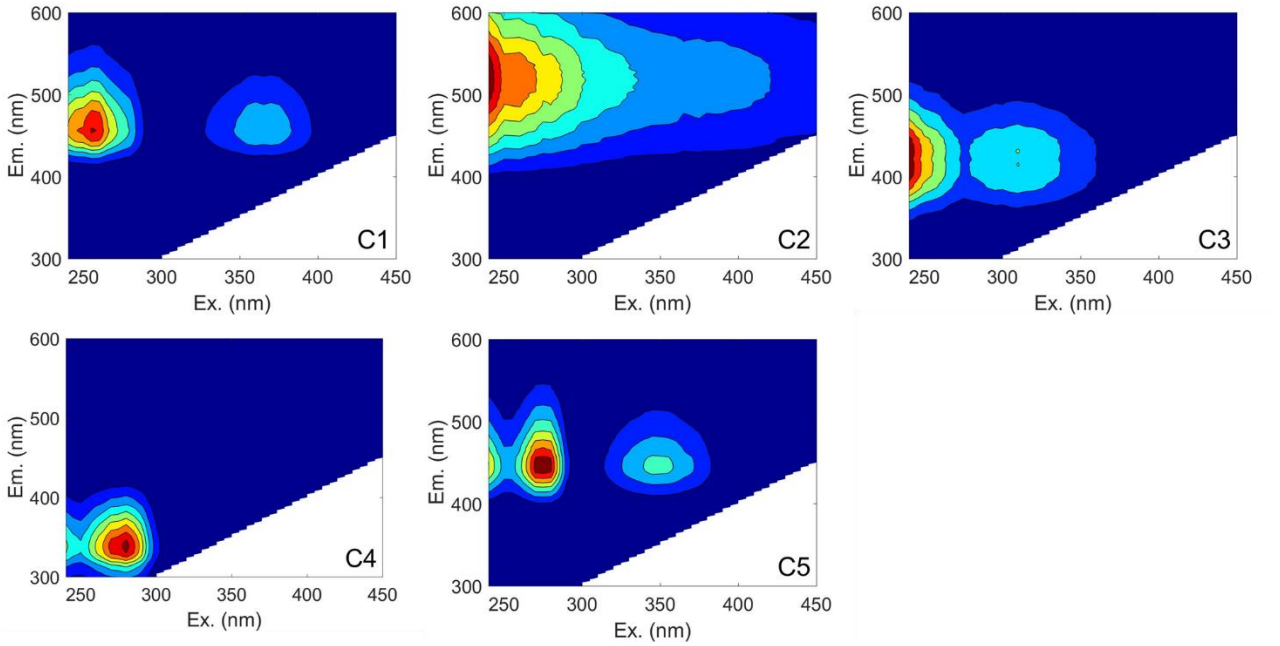

Figure 11. 5 component PARAFAC model identified for POM samples (n = 163). Model includes samples from both surface and bottom. The model was split half validated.

Table 4. Excitation maxima, emission maxima, the number of matches to OpenFluor and the assignment corresponding to previous studies for the 5 components identified. C5 matched in OpenFluor, but visually does not appear to match to the Sondergaard et al., 2003 study well. This component has been identified in POM+DOM PARAFAC models previously developed on bioassay samples using NRE water.

DOM Component	λ_{ex} (nm)	λ_{em} (nm)	Matches to OpenFluor	Assignment Corresponding to previous studies
C1	255, 360	456	2	Nutrient impacted estuaries; wastewater (Osburn et al., 2012); similar to ubiquinone (oxidized form of NADH), common in the BEPOM three-peak pattern, indicates recent biological activity (Brym et al., 2014)
C2	<240	518	25	Terrestrial, sediment fulvic-acid, humic like (Osburn et al., 2012; Brym et al., 2014); soil leachate (Osburn et al., 2016); high molecular weight (Søndergaard et al., 2003)
C3	<240, 310	430	26	Photolabile, found in agricultural dominated systems, terrestrial humic acid, possible photodegradation product (Osburn et al., 2012); extracted fulvic acids, modeled fluorescence for quinone-quinhydrone (Brym et al., 2014)
C4	<240, 280	338	12	Peak T, protein, tryptophan, recent biological production (Osburn et al., 2012; Brym et al., 2014; Osburn et al., 2016);
C5	<240, 275, 345	444	1	Humic C peak, terrestrial, humic-like (Søndergaard et al., 2003); uncharacterized

Sample residuals calculated after the application of the original POM PARAFAC model could not be modeled, indicating the original PARAFAC model accurately captured the fluorescence of all samples.

c. POM + DOM PARAFAC Model

A six component PARAFAC model was fitted to DOM (n = 471) and POM (n = 162) samples (Figure 12; Table 5). The model includes fluorescence signatures that are characteristic of both allochthonous and autochthonous sources. The model was split-half validated and all identified fluorescence components matched with previous studies on OpenFluor.

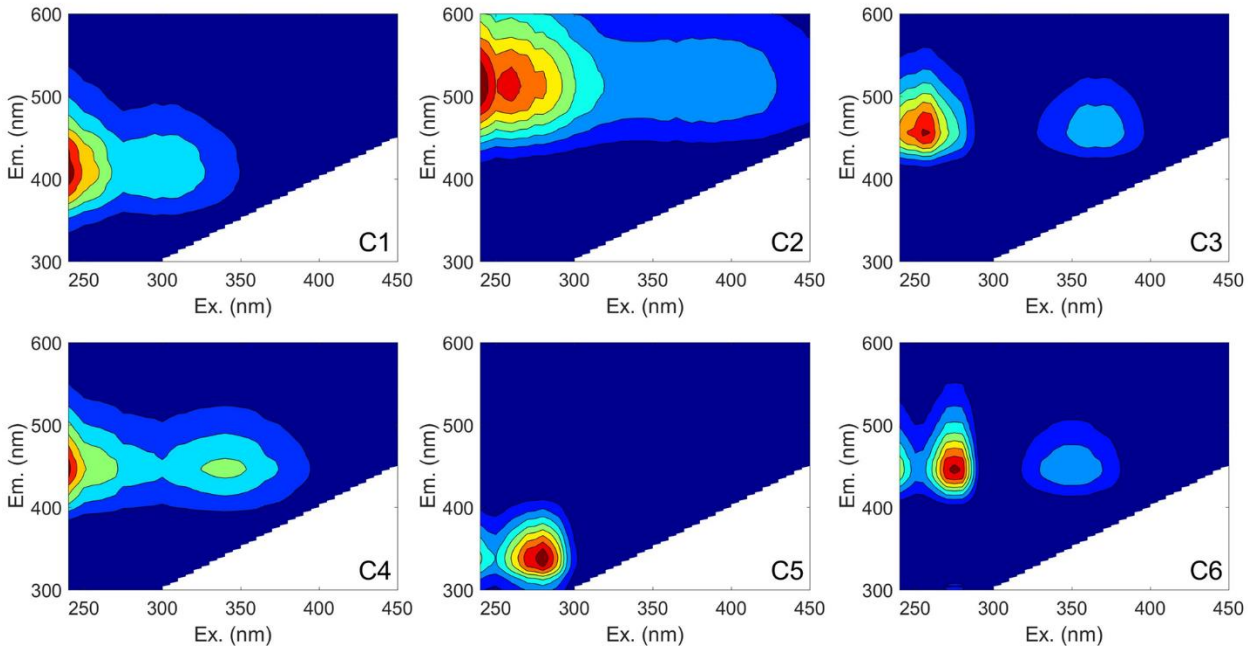

Figure 12. 6 component PARAFAC model developed on DOM (n = 471) and POM samples (n = 163). Model was split half validated.

Table 5. Excitation maxima, emission maxima, the number of matches to OpenFluor and the assignment corresponding to previous studies for all 6 components identified in the POM+DOM PARAFAC model.

DOM Component	λ_{ex} (nm)	λ_{em} (nm)	Matches to OpenFluor	Assignment Corresponding to previous studies
C1	<240, 295	410	20	Possible photodegradation product, eutrophic estuaries (Osburn et al., 2012)
C2	<240	512	22	Soil fulvic peak, high molecular weight (Brym et al., 2014; Kowalczuk et al., 2010; Osburn et al., 2012; Søndergaard et al., 2003); soil leachate (Osburn et al., 2016); humic-like, terrestrial, possible photochemical intermediate for the breakdown of terrestrial OM (Murphy et al., 2014)
C3	255, 360	456	2	Nutrient impacted estuaries, wastewater, microbial re-processing of terrestrial DOM (Osburn et al., 2012); oxidized form of NADH, eutrophic estuaries with human influence (Brym et al., 2014)
C4	<240, 340	446	11	Terrestrial, humic-like, peak A (Kowalczuk et al., 2010); terrestrial, humic-like, photolabile (Osburn & Stedmon, 2011); humic-like, waters with high OM loading, dominate in forested watersheds, produced during the break down of lignin (Murphy et al., 2014)
C5	<240, 280	338	13	Tryptophan, recent biological production, peak T (Osburn et al., 2012; Brym et al., 2014; Osburn et al., 2016)
C6	<240, 275, 350	446	1	Humic C peak, terrestrial, humic-like (Søndergaard et al., 2003); uncharacterized; identified in previous model developed on NRE bioassay samples

A 1-component PARAFAC model was fitted to sample residuals from the original POM+DOM PARAFAC model (Figure 13; Table 6). The model was not split-half validated and did not match any previous components in the OpenFluor database. The component appears to be in the terrestrial, humic-like region of fluorescence.

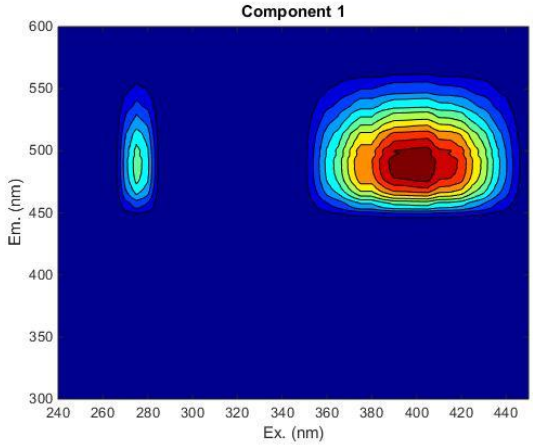

Figure 13. 1 component model developed on POM+DOM sample model residuals. The model was not split-half validated.

Table 6. Excitation maxima, emission maxima, and potential OM class assignment for the single component identified in the PARAFAC model fitted to POM+DOM model residuals. The component did not match with any models in OpenFluor.

DOM Component	Λ_{ex} (nm)	Λ_{em} (nm)	Potential OM class assignment
C1	275, 405	492	Appears humic-like, terrestrial in nature

3.3 Model Comparisons

The Matlab script TuckMatch was used to determine if components identified in the three developed PARAFAC models (DOM model, POM model, and POM+DOM model) were statistically similar (> 95% similarity) (Table 7) (Lorenzo-Seva and Berge, 2006). Comparisons are based on Tucker Congruence Coefficients.

Table 7. Model comparisons between the three PARAFAC models generated for samples collected from the NRE: the 3-comopnent DOM model, 5-component POM model, and 6-component POM+DOM model. An x indicates the components were > 95% similar.

	DOM C1	DOM C2	DOM C3	POM C1	POM C2	POM C3	POM C4	POM C5
POM C1								
POM C2								
POM C3		X						
POM C4								
POM C5								
POM+DOM C1		X				X		
POM+DOM C2					X			
POM+DOM C3				X				
POM+DOM C4	X							
POM+DOM C5							X	
POM+DOM C6								X

Based on model comparisons, the combined POM+DOM model is mainly driven by POM sample fluorescence. This is likely because both the POM and POM+DOM model were able to capture more fluorescence variability compared to the DOM model. The POM+DOM model did match with two of the three DOM components identified. Since the POM+DOM model appears to capture most POM and DOM fluorescence accurately, going forward, only the POM+DOM model will be used for analyses.

3.4 Biogeochemical parameters

The identified 6 components modeled in the POM+DOM PARAFAC model were plotted against various biogeochemical parameters measured by ModMon. By correlating identified OM components with nutrient, chlorophyll-a, and other chemical and physical parameters, a better understanding of the composition and bio-reactivity of these OM components can be determined. For this study, it is assumed that if phytoplankton are using OM sources, they are using this source as a nutrient source (nitrogen, N) and not as a carbon source (Kirchman, 2011).

The 6 PARAFAC components identified in the POM+DOM model were applied to the collected DOM samples and plotted against salinity (Figure 14; Table 8). Two general patterns emerge: 1. A two-end member mixing model where fluorescent intensity (i.e., concentration) decreases linearly with salinity. This represents a 1:1 mixing of the more fluorescent riverine end member (Neuse River) with the less fluorescent marine end member (Pamlico Sound) and 2. Fluorescent intensity either remains constant or increases down estuary, indicating that estuarine or marine, as opposed to dilution processes dominate the distribution of fluorescent intensity (Markager et al., 2011).

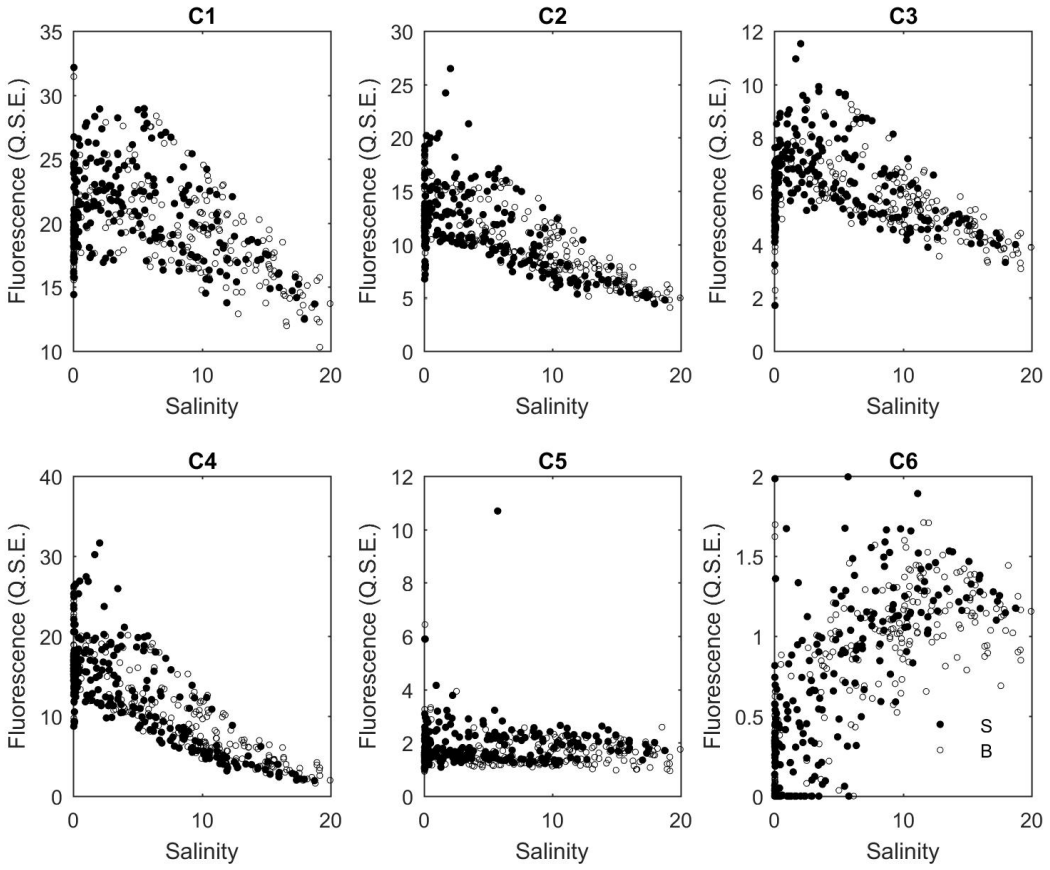

Figure 14. POM+DOM PARAFAC model components applied to DOM samples plotted for both surface (black circles) and bottom (open circles) versus salinity.

Table 8. ρ and p-values for correlations between the 6 PARAFAC identified components applied to DOM samples and salinity for both surface and bottom. n.s. indicates a non-significant result.

			Surface			
	C1	C2	C3	C4	C5	C6
ρ	-0.2225	-0.5872	-0.1799	-0.7155	0.1190	0.7336
R	<0.005	<0.005	<0.01	<0.005	n.s.	<0.005
			Bottom			
ρ	-0.4745	-0.8031	-0.4148	-0.8888	0.0142	0.7129
R	<0.005	<0.005	<0.005	<0.005	n.s.	<0.005

For DOM samples, C1, C2, C3, and C4 follow the two end-member mixing model. These components are typically thought of as terrestrial, humic-like fluorophores that are more concentrated in the riverine end-member and much lower in the marine end-member (Jaffé et al., 2014). C5 and C6, however, follow the second pattern and are dominated by estuarine processes. Fluorescent intensity of C5 remains constant with salinity indicating there is neither net consumption nor production of this component and the concentrations of this fluorophore are roughly equal in both the riverine and marine end members. C5 was identified as a protein, tryptophan fluorophore, which is known to be both produced and consumed by phytoplankton and microbial assemblages as well as photochemical processes in estuarine systems as

demonstrated in laboratory degradation studies (Chen & Jaffe, 2016). Protein-like, tyrosine fluorescence has been shown to have no relationship with salinity (Jaffe et al., 2014). Results from the current study demonstrate a similar pattern for the protein, tryptophan.

C6 increased with salinity indicating the marine end member contains higher fluorescent intensities compared to the riverine end member. This type of relationship with salinity has been demonstrated with other bio-reactive fluorophores including proteins (tryptophan, tyrosine), as well as the M-peak characterized as recent autochthonous, microbial production (Jaffe et al., 2014). In this study, C6 is a component that matched in OpenFluor, but still remains largely uncharacterized. Results from this study indicate C6 may be linked to recent autochthonous production in the estuary and/or is associated with more marine end-member water. This component was identified in an EEM-PARAFAC model developed for samples collected from a nutrient addition bioassay conducted in the NRE and may represent a bioreactive fraction of the OM pool (Peierls et al., unpublished).

The two components (C5 and C6) that were dominated by estuarine processes were plotted against chlorophyll-a (Figure 15; Table 9). By plotting these components against chlorophyll-a, an idea of the bio-reactivity of these components can be obtained. For surface samples, there was an apparent outlier in the dataset (chl-a > 400 µg L^{-1}). This measurement occurred on September 29, 2015 which followed the passage of Hurricane Joaquin and associated low pressure systems ("Joaqui'easter") that resulted in extreme precipitation over the NRE and NRE watershed. Following this event, a large phytoplankton bloom was observed at station 30 surface which resulted in the chlorophyll-a outlier. Two statistical analyses were conducted: one that included this outlier and an analysis that omitted this outlier. Visually, this magnitude of phytoplankton standing stock (as measured by chlorophyll-a) is associated with maximum fluorescence values for C5, indicating this component may be produced by phytoplankton. This relationship is less obvious for C6, indicating the origin of this component may not be from primary production.

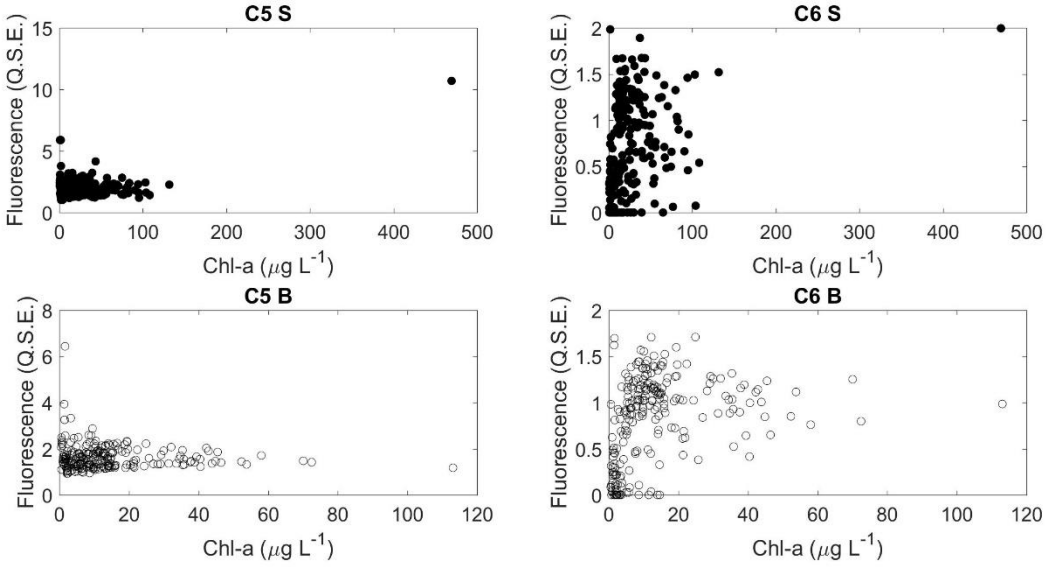

Figure 15. Component C5 and C6 applied to DOM samples for both surface (top graphs; black circles) and bottom (bottom graphs; open circles) plotted against chlorophyll-a.

Table 9. ρ and p-values for correlations between C5 and C6 applied to NRE DOM samples versus chlorophyll-a. With outlier indicates correlations that included the post-Joaqui'easter outlier; without outlier are correlations conducted without the post-Joaqui'ester outlier removed. n.s. indicates a non-significant result.

	Surface			
	With Outlier		Without Outlier	
	C5	C6	C5	C6
ρ	0.1576	0.4557	0.1468	0.4487
p	<0.05	<0.005	<0.05	<0.005
	Bottom			
ρ	0.0004	0.4732	n/a	n/a
p	n.s.	<0.005	n/s	n/a

The correlations with and without the post-Joaqui'easter outlier were not different. For surface samples, both C5 and C6 were positively correlated with chlorophyll-a, indicating these components are linked to recent primary production as being produced, consumed, or produced and consumed by phytoplankton and associated microbial assemblages. Based on the single outlier, it does appear that C5 (protein, tryptophan) is largely produced by phytoplankton assemblages. For bottom samples, only C6 had a statistically significant positive relationship with chlorophyll-a. Surface samples are largely associated with chlorophyll-a production, and therefore, it is assumed bottom samples would exhibit a less robust correlation with chlorophyll-a. Future analyses correlating chlorophyll-a with identified PARAFAC components will omit bottom samples.

The POM+DOM PARAFAC model was also applied to POM samples from both surface and bottom locations and plotted against salinity (Figure 16; Table 10). C1, C2, and C4 were negatively correlated with salinity. As with the DOM samples, these three components are considered terrestrial, humic-like fluorophores and would be expected to decrease down estuary as the riverine end member water is diluted by the marine end member (Jaffe et al., 2014).

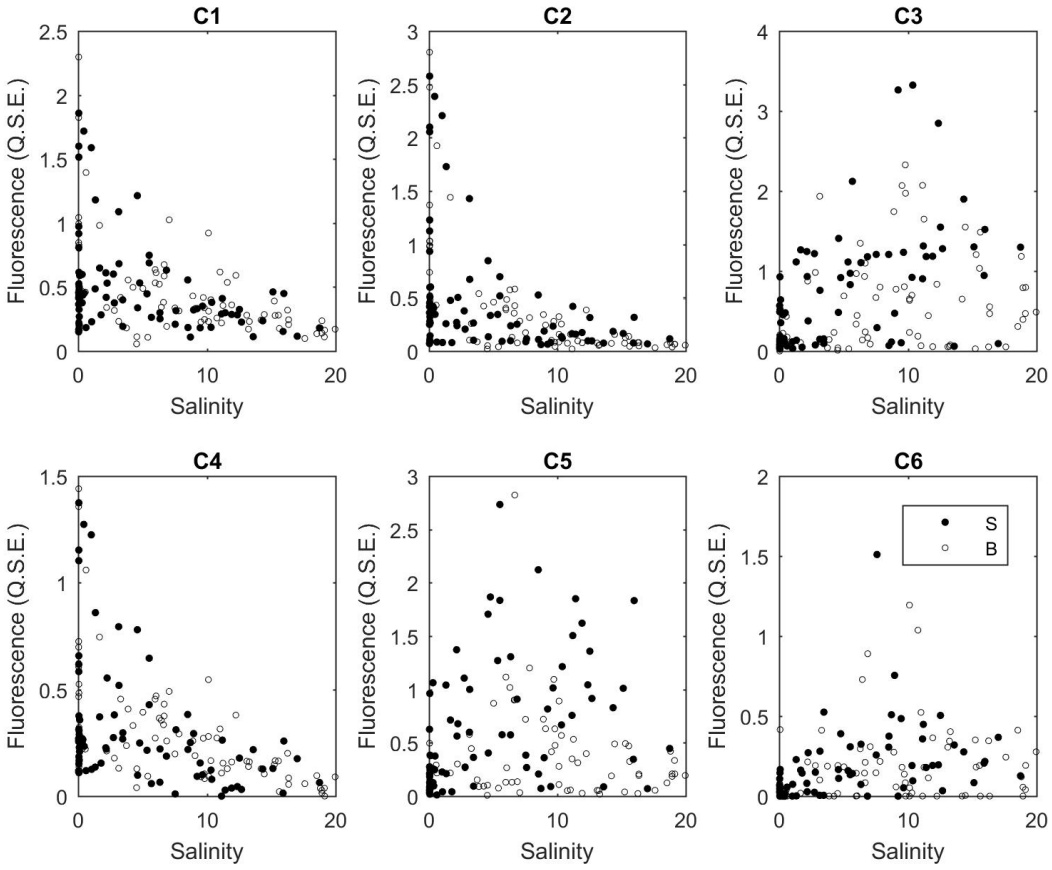

Figure 16. 6-component PARAFAC model applied to POM samples for both surface (black circles) and bottom (open circles) plotted against salinity.

Table 10. ρ and p-values for correlations between the 6 PARAFAC identified components applied to POM samples and salinity for both surface and bottom. n.s. indicates a non-significant result.

			Surface			
	C1	C2	C3	C4	C5	C6
ρ	-0.3825	-0.4436	0.5714	-0.4487	0.5304	0.6215
R	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
			Bottom			
ρ	-0.5547	-0.7014	0.3610	-0.6730	0.1345	0.2570
R	<0.005	<0.005	<0.005	<0.005	n.s.	<0.05

Unlike the DOM samples, C3 identified in the POM samples increased with salinity. C3 is associated primarily with POM samples and is characteristic of the POM 'three-peak pattern' (Brym et al., 2014). The positive correlation between C3 and salinity indicates this component is produced in the estuary and exists at higher concentrations in the marine end member (Markager et al., 2011). The differing relationships between DOM and POM samples for this component indicate this component has different sources within the estuary despite being identified as the same fluorophore. For DOM samples, this component is terrestrial and conservative in nature while the POM fraction is autochthonous and non-conservative. This discrepancy highlights the need for both the DOM and POM fractions to be measured

in order to fully understand how OM is being produced, consumed, and cycled in aquatic systems. Similar to DOM samples, C5 in surface samples and C6 in surface and bottom samples increased with salinity. As discussed above, this could be an indication that both of these components (C5, protein, tryptophan; C6, uncharacterized) are bio-reactive sources that are produced by phytoplankton and microbial assemblages in-situ, but not consumed.

The three components identified as being positively correlated with salinity (C3, C5, C6) were plotted against chlorophyll-a (Figure 17; Table 11). All three components were positively correlated with chlorophyll-a indicating these components are biologically active. These relationships appear to be more strongly correlated for the POM than DOM samples. POM fluorescence is often associated with chlorophyll-a and primary productivity and is therefore, expected to have stronger relationships with chlorophyll-a (Brym et al., 2014; Kirchman, 2011).

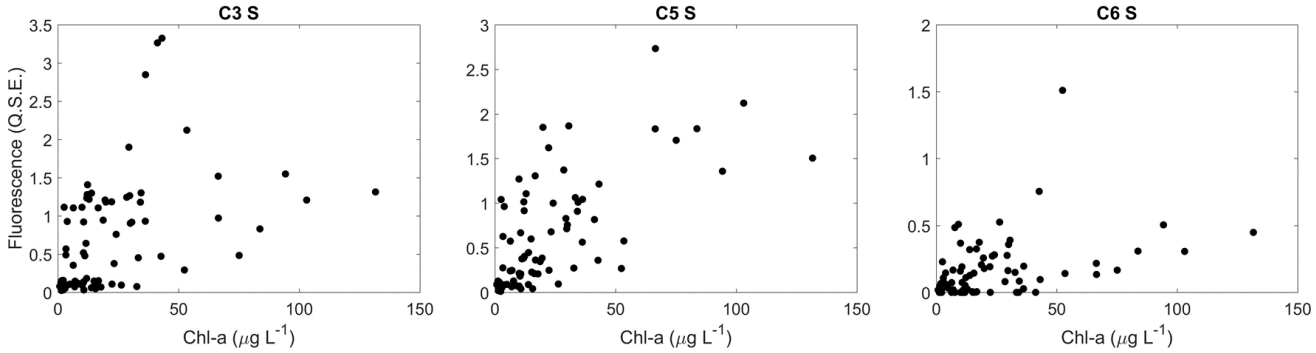

Figure 17. Components 3, 5, and 6 applied to POM surface samples plotted against chlorophyll-a.

Table 11. ρ and p-values for correlations between C3, C5, and C6 applied to NRE POM surface samples versus chlorophyll-a.

	Surface		
	C3	C5	C6
ρ	0.5712	0.6941	0.4433
p	<0.005	<0.005	<0.005

To gain a better understanding of the composition and nutrient quality of the identified fluorescent components, all 6 PARAFAC components as applied to DOM and POM samples were plotted against DON concentration (Figure 18; Table 12). For surface samples, as with chlorophyll-a, there was a noted outlier. This outlier corresponded to the sampling date following the Joaqui'easter and is likely a reflection of the higher than normal discharge measurements. As with the chlorophyll-a data, correlations were analyzed with and without this outlier. The outlier did not statistically alter any correlations between the fluorescent components and DON concentrations and results not including the outlier are omitted.

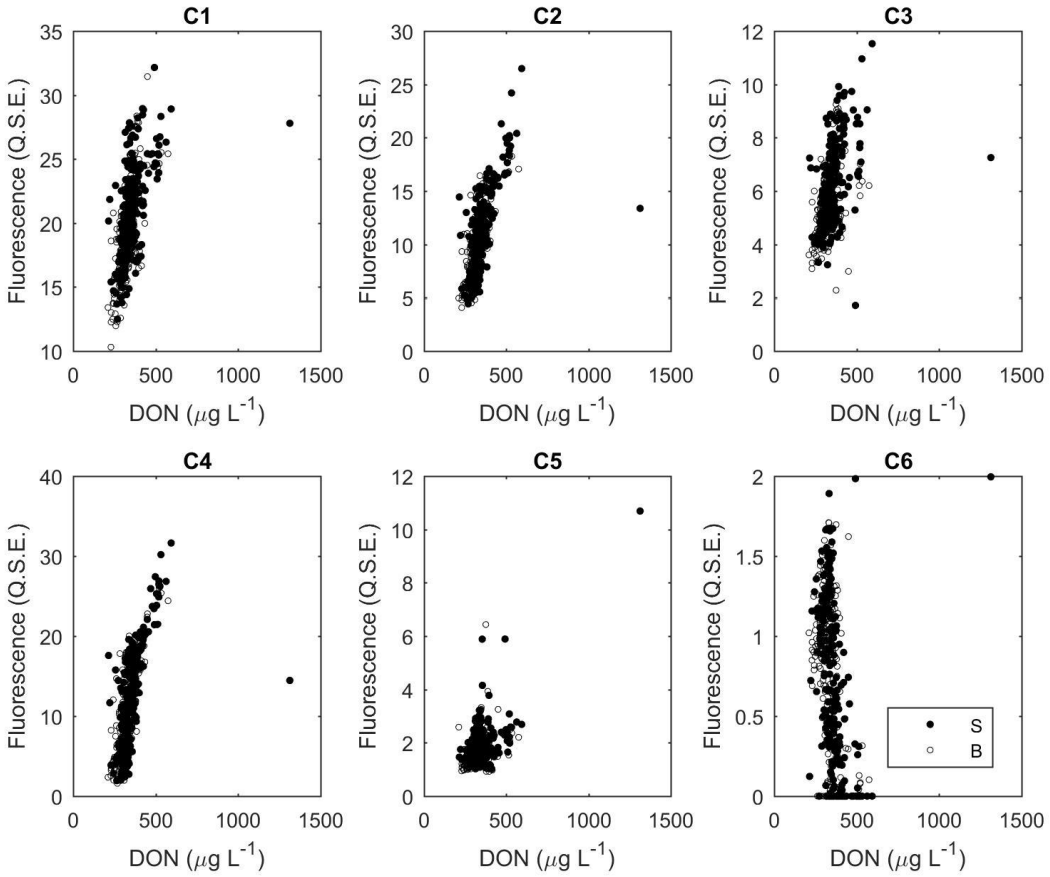

Figure 18. The 6 identified PARAFAC components applied to both surface and bottom DOM samples plotted against DON concentration.

Table 12. ρ and p-values for correlations between the 6 PARAFAC components for both surface and bottom DOM samples with DON.

	C1	C2	C3	C4	C5	C6
			Surface			
ρ	0.5929	0.7356	0.5605	0.7446	0.2575	-0.5193
p	<0.0005	<0.005	<0.005	<0.005	<0.005	<0.005
			Bottom			
ρ	0.6660	0.7658	0.5598	0.7564	0.3418	-0.3927
p	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005

For both surface and bottom, C1, C2, C3, C4, and C5 were positively correlated with DON concentration. This indicates these 5 OM fluorescent signatures contain N within their structure. Based on correlations with chlorophyll-a, it appears that only C5 and C6 may contain DON that is bio-reactive and potentially available to phytoplankton as a nutrient source.

C6 (un-characterized) had a statistically significant negative correlation with DON concentration. Based on correlations with chlorophyll-a, this component was considered bio-reactive and potentially produced by phytoplankton and associated microbial assemblages in-situ. It appears this autochthonously produced component is N-poor. This component also increased in intensity with salinity, indicating this component

is more abundant in the marine end-member water than the riverine water and is produced in-situ. Based on the available data, it appears this component is refractory and, while produced by in situ processes, cannot be consumed be phytoplankton or microbial assemblages.

PCA was conducted for both DOM and POM samples separately (Osburn et al., 2012; Brym et al., 2014). The POM+DOM PARAFAC model was applied to all samples. Each PARAFAC component was treated as a separate variable as was the fluorescence maximum for the particulate and dissolved fractions of each sample. Therefore, C1d refers to the C1 PARAFAC component for the dissolved fraction while C1p refers to the same C1 PARAFAC component but for the particulate fraction.

Results from the PCA conducted on the dissolved samples are shown in Figure 19. The two main axes captured about 73% of the variation in the samples. Principal component axis 1 (PC1) seems to be related to salinity such that variables associated with the higher salinity, marine end member have negative loadings and those associated with the lower salinity, freshwater end member have positive loadings. This reflects many of the correlations above, where C1, C2, C3, and C4 were identified as controlled by freshwater discharge while C6 increased in fluorescent intensity with increasing salinity and was reflective of autochthonous sources of DOM. Principal component axis 2 (PC2) appears to be divided largely based on chlorophyll-a. Positive loadings correspond to high chlorophyll-a and C5 fluorescent intensity while components C2 and C4 have negative loadings, further highlighting the terrestrial influence of these two components.

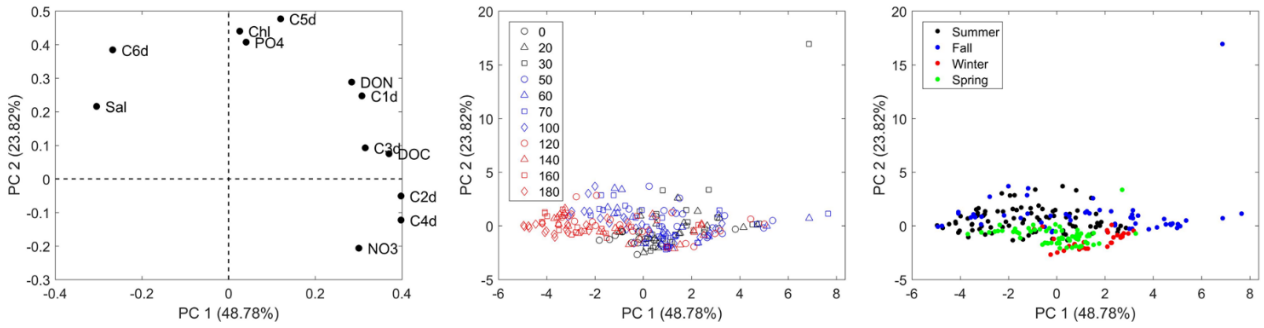

Figure 19. Results of the PCA for surface DOM samples. Loads for each variable are plotted in the left panel. Sample scores separated by station are plotted in the center, and sample scores separated by season are plotted in the right panel.

These patterns are reflected when sample scores are plotted for the two principal component axes. Generally, samples from the lower estuary (stations 120 – 180) are clustered towards the negative PC1 loadings reflecting the higher salinity of these samples. Similarly, the sample corresponding to September 29, 2015 when chlorophyll-a values were abnormally high, has a very high, positive PC2 loading reflecting the influence of chlorophyll-a on this sample and is clustered closely with C5 which was also associated with the high chlorophyll-a measurements at this sampling date.

For the particulate fraction, salinity also appears to be a main driver for PC1, but where positive loadings indicate high salinity and negative loadings correspond to low salinity (Figure 20). As with the DOM fraction, C1, C2, and C4 cluster towards the low salinity end and are indicative of more terrestrially derived POM. C6 is clustered near salinity indicating the more autochthonous, marine nature of this component. C5 also clusters with salinity and appears to be linked with recent, autochthonous POM production. PC2 is highly influenced by components C1, C2, and C4 and may be separating samples based on complexity of the molecular structure where C1, C2, and C4, are large, complicated molecules contrasted with C6 which may be a relatively small, simpler molecule.

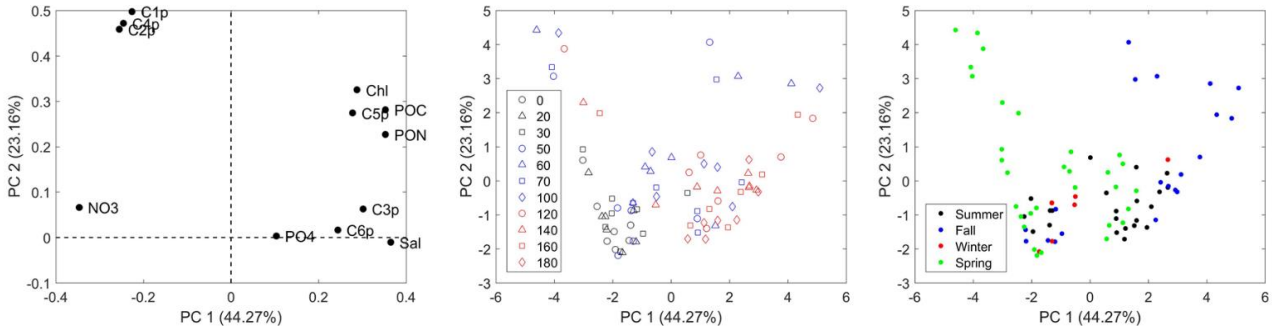

Figure 20. Results of the PCA for surface POM samples. Loads for each variable are plotted on the left panel. Sample scores separated by station are plotted in the center, and sample scores separated by season are plotted in the right panel.

For sample scores, upper estuary samples (station 0 – 30) are generally clustered in the bottom left hand corner and are indicative of low salinity. The samples that contain the most complex molecular structure are the mid-estuarine samples clustered in the upper left corner of the graph.

Comparing results and characteristics of the DOM and POM pools, there are noticeable similarities and differences. From a modeling perspective, the POM pool dominated the PARAFAC model and contained a higher proportion of components that are thought to be biologically reactive (Kirchman, 2011). The DOM pool was dominated by terrestrial components and was not able to fully capture all fluorescence variability in the samples. For this study, including the POM samples in the sampling regime allowed for a more detailed look at the sources of autochthonous as well as allochothonous sources of the entire OM pool.

The dominance of biological components for the POM samples reflects the coupling between chlorophyll-a, used as a proxy for phytoplankton standing stock, and the POM phase. Phytoplankton constitute a large fraction of the POM pool, particularly in the mid and lower estuary. Therefore, it is expected that the POM pool is mainly dominated by recent autochthonous OM production, as has been concluded in previous POM studies (Brym et al., 2014; Kirchman, 2011).

The DOM pool contains mainly terrestrial OM material that is largely refractory and diluted through the estuary by the clearer, less DOM rich marine waters. The biological components that may make up the DOM pool are probably much more difficult to capture in a given snap-shot based on the low concentrations and short time scales with which these components exist (Repeta, 2015; Sipler and Bronk, 2015). It is possible the DOM pool contains just as many biologically reactive component as the POM pool, as potentially indicated by the DOM residual PARAFAC model, but the fast turn-over time makes it difficult to capture these bioreactive components (McCallister et al., 2006; Stedmon & Cory, 2014).

Linking the POM and DOM pool, and components within these two pools, is difficult. Most of the components identified in the current study had similar patterns through the estuary for both OM pools. C1, C2, and C4 are considered terrestrial and decreased with salinity in a dilution, mixing model for both pools. Similarly, both C5 and C6 were considered biologically reactive and either remained roughly the same concentration with salinity (C5) or increased with salinity (C6) for both POM and DOM samples. C3 did exhibit different patterns for the POM and DOM pool and may indicate how these two pools are interacting. C3 was identified as a component common in eutrophic estuaries and may be a signal for microbial processing of DOM. This component is also commonly identified in POM samples and is characteristic of the BEPOM ‘three-peak’ pattern (Brym et al., 2014). For the DOM pool, this component decreased down estuary while for the POM pool this component increased. It is possible this indicates an

interaction between these two pools where C3 in the DOM pool is being degraded and transformed down estuary and is fueling production of C3 in the POM pool. Alternatively, these pools could be decoupled such that C3 in the DOM pool is simply being diluted by the marine end-member water (Jaffe et al., 2014) while C3 is being produced by the POM pool via primary production as fueled by inorganic nutrients in the estuary (Paerl et al., 2014). Teasing apart how these two pools interact in-situ is difficult and is confounded by various other processes occurring in the estuary.

3.5 Application of FluorMod

a. FluorMod Mixing Model

The FluorMod mixing model developed by Osburn et al., 2016 was applied to DOM samples collected in the NRE (n = 471). Because FluorMod was developed on DOM watershed samples, the model will not be able to fully capture the fluorescence variability of POM samples and therefore, discussion of FluorMod will be limited to DOM samples. DOM NRE samples were dominated by the reference (stream background; constituted ~80% of fluorescence) and soil leachate signal (constituted ~20-40% of fluorescence). This reflects the dominance of terrestrial, humic-like components in the DOM PARAFAC model discussed above. The 8 identified FluorMod sources are plotted against salinity for all DOM samples (Figure 21).

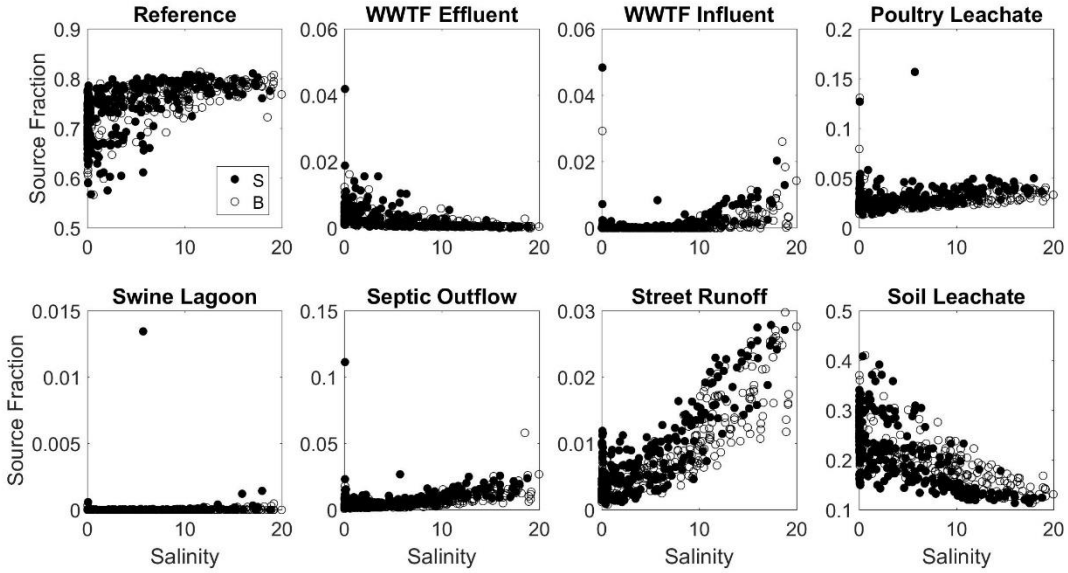

Figure 21. Plots of the 8 identified FluorMod sources as applied to DOM samples plotted against salinity for all surface (black circles) and bottom (open circles) samples.

Table 13. Table of correlations between the 8 identified FluorMod sources as applied to DOM estuarine samples and salinity for both surface and bottom samples.

	Reference	WWTF Effluent	WWTF Influent	Poultry Leachate	Swine Lagoon	Septic Outflow	Street Runoff	Soil Leachate
				Surface				
ρ	0.6481	-0.6340	0.5158	0.4577	0.1774	0.6557	0.7403	-0.6731
p	<0.005	<0.005	<0.005	<0.005	<0.01	<0.005	<0.005	<0.005
				Bottom				
ρ	0.7409	-0.6835	0.6472	0.5261	0.1534	0.8037	0.8848	-0.8080
p	<0.005	<0.005	<0.005	<0.005	<0.05	<0.005	<0.005	<0.005

The 8 FluorMod sources identified in each DOM sample were correlated against salinity for both surface and bottom (Table 13). WWTF effluent and soil leachate source fractions were negatively correlated with salinity and follow a mixing pattern such that the DOM rich, dark river water is diluted by the DOM poor marine waters of the Pamlico Sound. This pattern is expected for the soil leachate as this source is considered largely refractory and is abundant in riverine water, but represents a much smaller portion of the DOM pool in more marine waters (Markager et al., 2011).

The remaining 6 FluorMod sources identified are positively correlated with salinity. Some of these sources might be expected to increase down estuary as land use changes from the more urban upper estuary to the more rural mid to lower estuary. This includes sources such as poultry leachate and septic outflow. Both of these sources, however, contain a large fraction of protein-like fluorescence which is not only derived from these terrestrial, allochthonous sources, but can also be produced in-situ by phytoplankton and microbial assemblages. It is possible these sources may be modeling and capturing fluorescence from these autochthonous processes. It is not necessarily possible in aquatic systems to correctly identify the source of protein-like fluorescence and accurately assign this component as either allochthonous (as from poultry waste and septic systems) or autochthonous (as phytoplankton or microbial exudates) sources without more detailed geochemical analyses (Kaplan & Cory, 2016).

Other identified FluorMod sources would not be expected to increase with salinity. This includes the reference signal and the street run-off signal. The reference signal was characterized by Osburn et al., 2016 as the background OM signal inherent to streams that drain largely forested or 'natural' watersheds. It is generally expected that the background, terrestrial OM signal decreases down estuary in a mixing pattern as described above. However, it appears that the reference signal is pervasive throughout the estuary such that this component may be completely refractory and exists in essentially equal concentrations in both the riverine and marine end members. The street runoff source was positively correlated with salinity as well. This is unexpected in an estuary that moves from urban to rural land use and indicates there is production of this source in the estuary. Based on these results, it is hypothesized the street runoff signal is being misrepresented within the estuary. Instead, the street run-off signal is assumed to represent some type of autochthonous fluorescence signal that is produced by phytoplankton and microbial assemblages in the NRE and Pamlico Sound, such that the fluorescence intensity of this signal increases down estuary in response to increasing phytoplankton and microbial production. Because the fluorescent intensity of this component increases down the estuary, it appears there is no removal process and this component is possibly resistant to degradation and utilization (Jaffe et al., 2014). Previously conducted studies have demonstrated phytoplankton and microbial assemblages are capable of producing refractory DOM and POM (Carlson and Hansell, 2015).

b. FluorMod PARAFAC Model

As noted previously, the FluorMod mixing model does not include any autochthonous sources. Looking at results from the application of FluorMod to estuarine samples, it appears the identified sources are not accurately characterizing some of the identified allochthonous sources (i.e., street runoff) because of the lack of autochthonous sources in the mixing model. In order to better identify and track both allochthonous and autochthonous sources, the PARAFAC model which the FluorMod mixing model was developed on was applied to DOM samples. The PARAFAC model contained both allochthonous and autochthonous fluorescence components and could be used to identify and track DOM production in the estuary.

In an effort to verify the ability of the FluorMod PARAFAC model to capture fluorescence variability in NRE DOM samples, sample residuals from the FluorMod PARAFAC model applied to NRE samples were modeled. A 1 component model was developed (Figure 22; Table 14). The model was not split-half validated and did not match with any previously identified components in OpenFluor. The identified

component is in the terrestrial, humic-like region of fluorescence. Because only a single residual component was identified, it appears the FluorMod PARAFAC model does a decent job of capturing fluorescence variability even in estuarine samples.

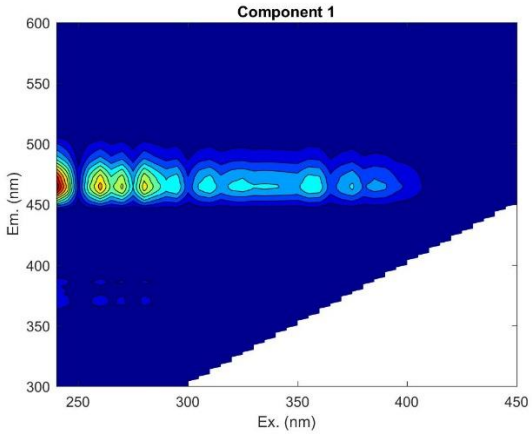

Figure 22. A one component model was generated based on sample residuals after application of the FluorMod PARAFAC model. The model was not split-half validated.

Table 14. Excitation maxima, emission maxima, and potential organic matter class assignment. The component did not match in OpenFluor.

Residual Component	Λ_{ex} (nm)	Λ_{em} (nm)	Potential organic matter class assignment
C1	<240 - 375	464	Terrestrial, humic-like fluorophore

The FluorMod PARAFAC model was applied to all DOM samples. The fluorescence of each component in each collected sample was plotted against salinity (Figure 23). The PARAFAC model contains 9-identified components, however, component 9 was zero for all samples and is omitted from any further analysis. DOC is included for reference.

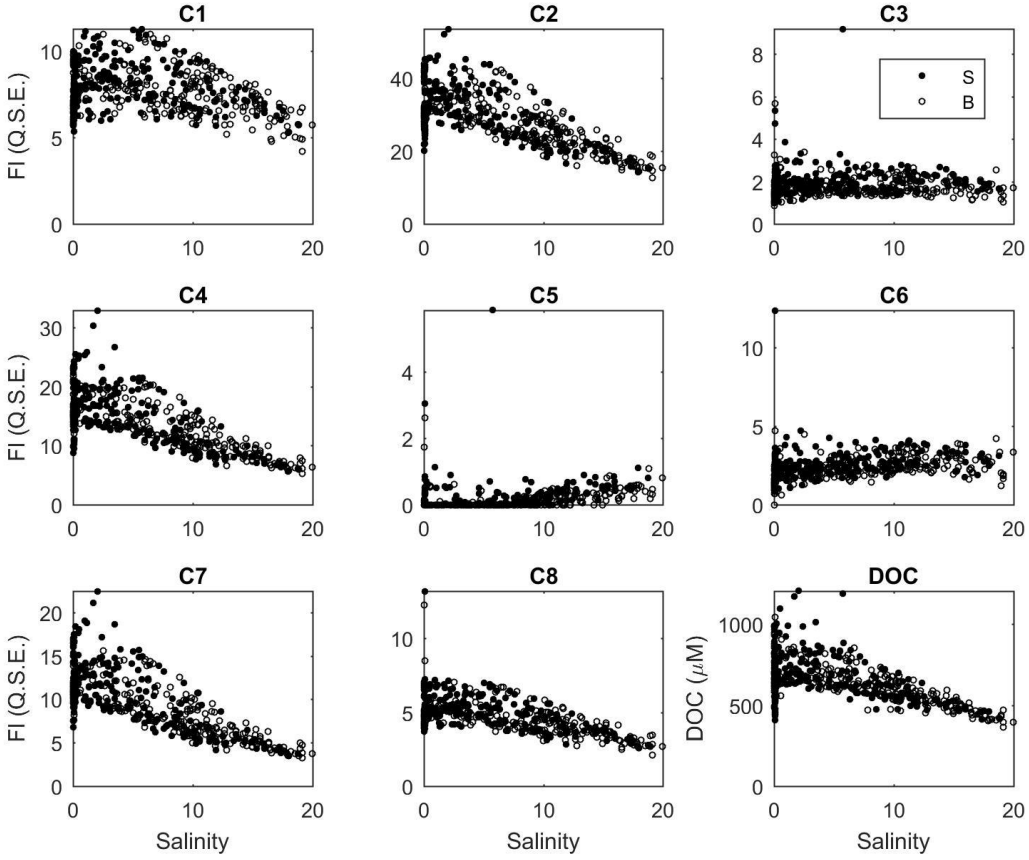

Figure 23. Plots of the first 8 identified components in the FluorMod PARAFAC model applied to samples collected in the NRE plotted against salinity for surface (black circles) and bottom (open circles). DOC is included.

Table 15. ρ and p values for correlations between the 8 identified PARAFAC components and DOC versus salinity for both surface and bottom samples. n.s. indicates a non-significant result.

					Surface				
	C1	C2	C3	C4	C5	C6	C7	C8	DOC
ρ	-0.0396	-0.5213	0.3179	-0.5872	0.4957	0.5218	-0.6241	-0.4005	-0.4304
p	n.s.	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005
					Bottom				
ρ	-0.2751	-0.7655	0.1790	-0.8102	0.6397	0.4838	-0.8439	-0.6641	-0.7343
p	<0.005	<0.005	<0.01	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005

Two different patterns appear in the data when components are correlated against salinity (Table 15). The first pattern is characterized by mixing where riverine water is diluted by the marine end-member water (Markager et al., 2011). Components that follow this pattern are those that are typically considered terrestrial, humic-like and include C1 (leaf material), C2 (natural DOM in streams), C4 (soil leachate), C7 (effluent-like), C8 (microbial activity) and DOC. Most of these components (C1, C2, C4, C5, DOC) are expected to follow this pattern as they are dominant in riverine environments heavily influenced by

terrestrial DOM and decrease as the riverine water mixes with the DOM poor, clearer marine waters. The microbial activity component (C8) is not expected to follow the strict mixing pattern as bacteria produce DOM in-situ. Previous studies have concluded this microbial, M-peak may represent both terrestrial and microbial processes and therefore, is not necessarily a clear indicator of recent, in-situ microbial activity (Murphy et al., 2008; Stedmon & Cory, 2014).

The second pattern is characteristic of estuarine processes, such that the fluorescent intensity of the component increases with salinity. This includes components C3 (protein, tryptophan), C5 (protein, tyrosine), and C6 (urban run-off). A similar pattern was observed for two identified protein components in an Agro-Urban estuary in Australia where they determined production of these components in the system, via phytoplankton and bacterial assemblage exudates (Fellman et al., 2011). As with the street runoff source modeled in the FluorMod mixing model, the fluorescence component identified as urban runoff increased with salinity. As explained previously, it is believed this component is not accurately identified in the estuary. A similar component has been preliminarily identified as a potentially biologically active, phytoplankton signal in a cyanobacterial dominated freshwater system (Hounshell et al., unpublished results).

The three components following estuarine processes (C3, C5, and C6) were plotted against surface chlorophyll-a concentrations measured in the NRE (Figure 24; Table 16). There is an abnormally high chlorophyll-a measurement that corresponds to an intense phytoplankton bloom observed at Station 30 the 'Joaqui'easter' as described previously. Two sets of correlations were analyzed: one that included this outlier and an analysis that omitted this outlier.

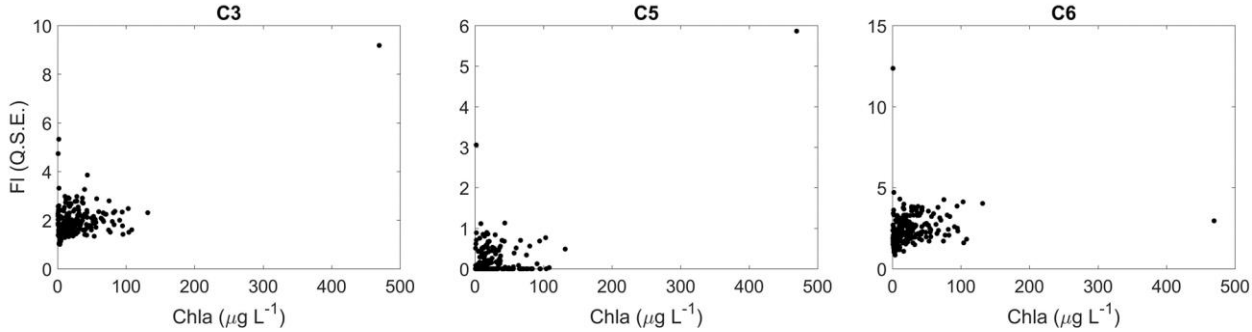

Figure 24. Plot of the three FluorMod PARAFAC components following estuarine dominated processes plotted against salinity.

Table 16. ρ and p-values for correlations between estuarine dominated components (C3, C5, C6) and chlorophyll-a concentrations with and without the identified chlorophyll-a outlier. n.s. indicates a non-significant result.

	Surface					
	With Outlier			Without Outlier		
	C3	C5	C6	C3	C5	C6
ρ	0.3010	0.1361	0.3906	0.2921	0.1239	0.3871
p	<0.005	<0.05	<0.005	<0.005	n.s.	<0.005

The abnormal chlorophyll-a value after the Joaqui'easter does have an influence on the correlations between some of the components and chlorophyll-a, primarily for C3 and C5, the two protein signals. Fluorescence intensity for both of these components are nearly double when chlorophyll-a is ~400 µg/L,

indicating these components may be derived from phytoplankton assemblages. Both C3 and C6 are positively correlated with chlorophyll-a indicating these two components are bioreactive and are most likely produced by phytoplankton and microbial assemblages.

4. Discussion

4.1 What is the extent of transport and the fate of natural and anthropogenic PON and DON sources in the N-sensitive NRE?

There are several ways the transport and fate of PON and DON sources in the NRE can be assessed using results from this study including using the POM+DOM PARAFAC model developed on samples collected as well as assessing results of FluorMod applied both as a mixing model and a PARAFAC model to DOM samples. By looking at how these various identified components change with salinity, an idea of the source and fate of these components can be determined. A summary of these relationships are listed in Table 17.

Table 17. Summary of mixing patterns observed for DOM and POM samples for the combined POM+DOM model developed on NRE samples collected during this study and FluorMod for both the mixing model developed and the PARAFAC model. 'D' = dilution where concentrations decrease linearly with salinity. 'E' = estuarine where concentrations are either stable with salinity or increase as indicated.

Model and Component	Component designation	Mixing pattern DOM samples	Mixing pattern POM samples
POM+DOM C1	Photodegradation product, eutrophic estuaries; terrestrial	D	D
POM+DOM C2	Soil fulvic acid; terrestrial	D	D
POM+DOM C3	Nutrient impacted estuaries, wastewater, microbial re-processing of terrestrial DOM; terrestrial, conservative for DOM; bio-reactive, biologically produced for POM	D	E; increased w/ salinity
POM+DOM C4	Terrestrial, humic-like, peak A	D	D
POM+DOM C5	Protein – tryptophan; autochthonous, consumed in-situ	E; constant concentration	E; increased w/ salinity
POM+DOM C6	Uncharacterized; autochthonous, N-poor, refractory	E; increased w/ salinity	E; increased w/ salinity
FluorMod 1	Stream background	E; increased w/ salinity	
FluorMod 2	WWTF Effluent	D	
FluorMod 3	WWTF Influent	E; increased w/ salinity	
FluorMod 4	Poultry Leachate	E; increased w/ salinity	
FluorMod 5	Swine Lagoon	E; increased w/ salinity	
FluorMod 6	Septic outflow	E; increased w/ salinity	
FluorMod 7	Street runoff	E; increased w/ salinity	
FluorMod 8	Soil leachate	D	
FluorMod PARAFAC C1	Leaf material	D/E; constant concentration	
FluorMod PARAFAC C2	Natural DOM in streams	D	
FluorMod PARAFAC C3	Protein – tryptophan	E; increased w/ salinity	
FluorMod PARAFAC C4	Soil leachate	D	
FluorMod PARAFAC C5	Protein – tyrosine	E; increased w/ salinity	
FluorMod PARAFAC C6	Urban runoff	E; increased w/ salinity	
FluorMod PARAFAC C7	Effluent like	D	
FluorMod PARAFAC C8	Microbial activity	D	

Sources of DOM and POM to the NRE are largely terrestrial and decrease linearly with salinity, indicating they follow a dilution, or two end-member mixing pattern signifying these sources are refractory. The majority of components identified as un-reactive are those associated with large, complex, and refractory molecules that have been shown to exhibit similar trends through other estuarine systems (Jaffe et al., 2014). Other identified sources and components either remained constant through the estuary or increased with salinity, indicating processes other than mixing were occurring. These components were mainly identified as biological in nature (i.e., proteins or microbial associated components) and are assumed to be either produced or consumed by phytoplankton and microbial assemblages in-situ (Stedmon and Cory, 2014 and references therein). Many of these components, particularly the two protein sources, tryptophan and tyrosine, have been shown to exhibit similar patterns in other estuarine systems (Jaffe et al., 2014).

Patterns and results become more complicated to interpret when there are multiple sources of the same OM class. This is most likely true for the vast majority of OM classes identified, but is particularly difficult to assess for protein-like signatures that can come from terrestrial, watershed sources including WWTF effluent and animal waste, as well as produced by phytoplankton and bacterial assemblages in-situ (Kaplan and Cory, 2016). Teasing apart what fraction of the protein signal originated from watershed sources versus what was produced in-situ without more specific geochemical analyses is difficult.

4.2 What is the bioreactivity of ON through the estuarine-freshwater continuum? Are ON signatures changing in magnitude (i.e., concentration) through the NRE, indicating degradation or utilization by phytoplankton and microbial communities?

As described above, there are several sources and ultimate fates of both DOM and POM in estuarine environments, one of which includes production and/or consumption of OM by phytoplankton and microbial assemblages (Stedmon and Cory, 2014). Results from this study indicate phytoplankton and microbial assemblages do produce both DOM and POM in-situ and evidence that suggests these same assemblages may be using the OM pool as a nutrient source for growth.

There were several components and sources of both DOM and POM that increased with salinity and were associated with chlorophyll-a. This was particularly true for protein components, but also included components associated with recent microbial production. These relationships were strongest for POM components. A large portion of the POM pool is produced via phytoplankton primary production and therefore, it would be expected that many of these identified, biologically active POM components are strongly correlated with chlorophyll-a (Brym et al., 2014; Kirchman, 2011; Osburn et al., 2012). Components in the DOM pool were also correlated with chlorophyll-a and indicate exudates from phytoplankton and bacterial assemblages are also a source of DOM (Stedmon and Cory, 2014; Repeta, 2015).

There were two different patterns associated with estuarine processes: 1. Fluorescent intensity of components increased with salinity and 2. Fluorescent intensity of components remained constant with salinity (Markager et al., 2011). For components that increased with salinity, it could be assumed that these components, while produced by phytoplankton and microbial assemblages, are largely refractory as the concentration of the fluorescent intensity of these components is higher in the marine end member water where sources of autochthonous OM would dominate (Jaffe et al., 2014). This type of pattern was observed for POM samples identified as POM+DOM C3 and C5, as well as POM and DOM samples identified as POM+DOM C6. Estuarine processes where the fluorescent component intensity remains constant through the estuary may indicate there is some kind of control on this OM class, limiting the accumulation of this component (Stedmon and Cory, 2014). This could indicate that while the OM component is being produced in-situ, it is also being consumed. This type of pattern was observed for

DOM samples identified as POM+DOM C5 as well as DOM samples identified in the FluorMod PARAFAC model C3 and C6.

Based on this assertion, the protein and microbially associated OM classes contained within the DOM pool are largely considered to be both produced and consumed in-situ while the bio-reactive OM classes contained in the POM pool are largely produced in-situ, but not necessarily consumed. This reflects what is known about the two pools: mainly that the DOM pool is more readily used by phytoplankton and bacterial assemblages as it is in an easier form for phytoplankton and microbes to use while the POM pool is largely reflective of recent phytoplankton production but is difficult for phytoplankton and bacteria to access in the particulate phase (McCallister et al., 2006).

5. Conclusions

In order to fully understand OM and ON cycling within estuarine and aquatic systems, both the DOM and POM pool should be assessed. For this specific study, if only the DOM pool had been assessed, the seemingly 'bio-reactive' fractions of the OM pool would have gone largely unconstrained. The addition of the POM samples allowed for the identification and tracking of the OM pool biological fraction. This highlights some of the differences in the sources of these two pools: the DOM pool is largely dominated by terrestrial, allochthonous OM signals while the POM pool is largely dominated by autochthonous OM signals produced by phytoplankton and microbial assemblages (McCallister et al., 2006).

With respect to OM cycling within each respective pool (POM, DOM) there were similarities and differences based on the identified components. The PARAFAC components identified as terrestrial (C1, C2, C4) decreased with salinity for both fractions, indicating these humic-like, terrestrial components are refractory in both the DOM and POM pools (Jaffe et al., 2014). There were differences in OM cycling between the two fractions for C3. For the DOM pool, this component exhibited patterns with salinity similar to the terrestrial, conservative components described above. For the POM pool, the C3 component increased with salinity indicating this component in the POM pool is autochthonous and largely refractory once produced in the estuary. This C3 component has been identified in previous studies of POM in estuarine environments and is a critical component of the POM 'three-peak signature' (Brym et al., 2014). The same study found strong correlations between this C3 component and chlorophyll-a, and concluded this component is produced by phytoplankton and microbial assemblages in-situ (Brym et al., 2014).

For both the POM and DOM pools, C5 and C6, characterized as autochthonous, exhibited patterns dominated by estuarine processes. C6 was considered un-characterized and lacked convincing matches to previously identified components in OpenFluor. Results from this study, both in the DOM and POM pool, indicate this component is autochthonous, refractory, and N-poor. It is hypothesized this component contributes to the characteristic 'three-peak' POM pattern seen in estuarine and marine samples influenced by recent autochthonous production (Brym et al., 2014).

C5 was identified as the protein tryptophan and exhibited estuarine dominated process for both the DOM and POM pool. For the DOM pool, the fluorescent intensity of this component remained relatively stable through the estuary, indicating this component may be both produced and consumed in the estuary. Previous studies have concluded this component is produced in-situ and can also be consumed by phytoplankton and microbial assemblages, particularly when dissolved inorganic nutrient sources are depleted, as would be the case in the lower NRE (Stedmon and Cory, 2014). For the POM pool, the fluorescent intensity of this C5 component increased with salinity indicating this component is produced in the POM pool but remains largely refractory through the estuary (Jaffe et al., 2014). It is assumed the DOM pool is more easily accessible for phytoplankton and bacterial assemblages to use this potential DON source and therefore, while consumption may exist in the DOM pool it can be largely absent in the POM pool (McCallister et al., 2006).

6. Recommendations

Results from this study do indicate that sources of DON, primarily as protein, may be used as a nutrient source for phytoplankton and microbial assemblages in-situ. This has important implications for managing nutrient loading, particularly to N-sensitive estuarine and coastal systems. These results suggest both DIN sources of N as well as OM sources of N should be considered when establishing nutrient budgets to impaired systems, such as the NRE. Attention should particularly be paid to sources of DON that contain proteins (as tryptophan and tyrosine) including waste sources such as WWTF effluent, chicken litter, swine lagoon waste, septic outflow, and autochthonous sources produced by phytoplankton and bacterial assemblages in the estuary. The EEM-PARAFAC technique used in this study is unable to differentiate between these different protein sources in-situ. Therefore, it is recommended that more research be conducted to specifically track protein-like DON from the above mentioned sources to determine which watershed (or autochthonous) protein sources contribute to this bio-reactive pool in the estuary.

7. Dissemination of information

a. Communication of results

Presentations:

Hounshell, A.G., Osburn, C.L., Peierls, B.L., and Paerl, H.W. (2017). Role of organic nitrogen to eutrophication dynamics along the Neuse River Estuary, NC. Oral Presentation at WRRI Annual Conference, 16 March, Raleigh, NC.

Hounshell, A.G., Osburn, C.L., Peierls, B.L., and Paerl, H.W. (2017). Role of organic nitrogen to eutrophication dynamics along the Neuse River Estuary, NC. Poster Presentation at NC MarCo Student Symposium, 31 Mar, Beaufort, NC. *

* Presentation received 1st place for Student Poster Presentation

Blog posts:

A summary of the NRE and the issues associated with eutrophication and DON loading was communicated via an informal blog post.

Hounshell, Alexandria G. “The scientific method in real life”. Web blog post. *UNdertheC Blog*. 17 Jan. 2017. (https://underthecblog.org/page/2/)

A summary on the importance of water color to water quality in aquatic systems as well as a discussion and tutorial about the ‘Eye on Water’ app used to assess water color.

Hounshell, Alexandria G. “I’ve got my ‘Eye on Water’”. Web blog post. *UNdertheC Blog*. 13 Apr. 2017. (https://underthecblog.org/2017/04/13/i-got-my-eye-on-water/)

Websites:

A web page was generated for optical DOM and POM work conducted in the Neuse River Estuary, NC as part of this and other projects. The webpage details the importance of the work and the methods used.

Hounshell, Alexandria G. “Neuse River Estuary, North Carolina”. (http://ahounshell5.wixsite.com/research/neuse-river-nc)

A second web page was generated to discuss the importance of water color to water quality and to provide a tutorial about the ‘Eye on Water’ app.

Hounshell, Alexandria G. “Eye on Water”. (http://ahounshell5.wixsite.com/research/eye-on-water)

8. Summary

A year-long environmental survey was conducted in conjunction with the UNC-CH ModMon sampling program to collect and analyze DOM and POM sources, fates, and bio-reactivity in the NRE. Broad organic matter classes were identified from these samples and tracked both spatially and temporally through the estuarine system using the EEM-PARAFAC techinque. Additionally, FluorMod, a mixing model developed on watershed sources of DON to the Neuse River was used to identify and track watershed sources of DON in the estuary. Overall, OM in the NRE is largely composed of natural, terrestrial sources of OM. There were, however, some OM classes identified that are considered biologically reactive including components (as proteins and microbially produced components) that were both produced and consumed by phytoplankton and microbial assemblages in-situ. Results from this study indicate there may be bio-reactive fractions of the OM pool, both of which may be produced in the watershed or in-situ, and can be consumed by phytoplankton and microbial assemblages as a nutrient source. Future research is required to confirm the exact sources of the protein sources (as either allochthonous or autochthonous sources), however, this study does demonstrate the importance of the ON pool as a nutrient source for phytoplankton and microbial assemblages in the N-sensitive NRE.

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Particle-bound Nutrients in Stormflow: A New Approach for Monitoring and Predicting N and P Transport and Fate in Watersheds of the NC Piedmont

Basic Information

Title:	Particle-bound Nutrients in Stormflow: A New Approach for Monitoring and Predicting N and P Transport and Fate in Watersheds of the NC Piedmont
Project Number:	2016NC199B
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End Date:	2/28/2017
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Congressional District:	NC-001
Research Category:	Water Quality
Focus Category:	Nutrients, Sediments, Water Quality
Descriptors:	None
Principal Investigators:	Curtis J. Richardson, Mark River

Publications

There are no publications.

WRRI Progress Report

Project Title: Particle-Bound Nutrients in Stormflow: A New Approach for Monitoring and Predicting Nitrogen and Phosphorus Transport and Fate in Watersheds of the North Carolina Piedmont

Principal Investigator: Mark River

Contact info: email: mark.river@duke.edu

Date: 5/8/2017

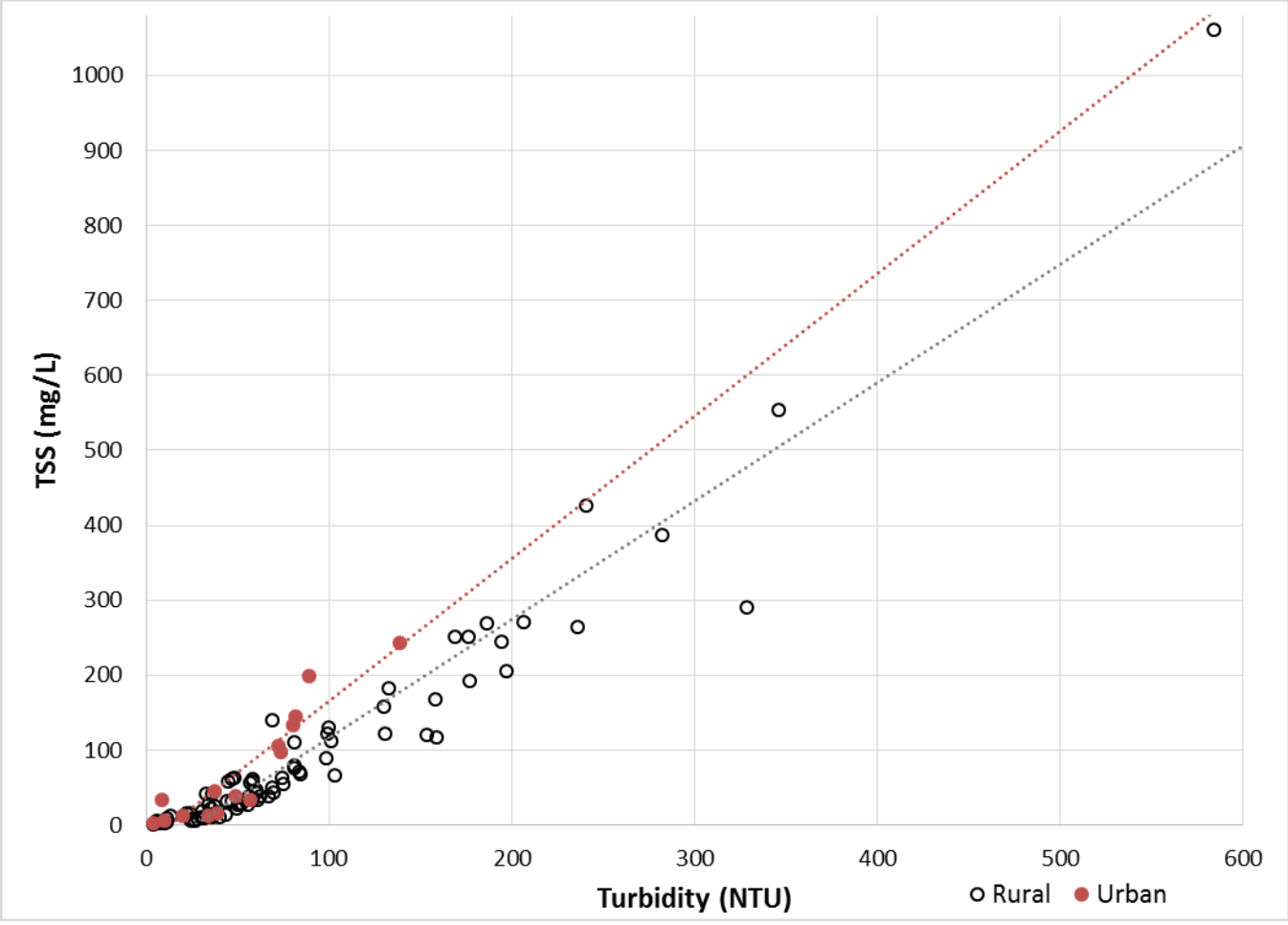
Activities to date:

We have to date analyzed numerous storms for turbidity, total suspended sediment, and total nutrients. We have measured the sized of particles in stormflow via flow-imaging particle size analyses along with counting particles "by hand" in images obtained via scanning electron microscopy. We have quantified the crystalline mineral components of stormflow via X-ray diffraction on filtered sediments.

Findings to date:

We have found a very strong relationship between turbidity and Total Suspended Sediments (TSS), for rural and urban streams in the central North Carolina Piedmont (figure 1).

Figure 1: Relationship between turbidity and suspended sediments, for rural and urban streams in the central NC Piedmont.



The particle size distribution is skewed towards particles less than one micron in diameter, measured both by flow-imaging particle size analysis (figures 2 and 3) and confirmed by counting particles via Scanning Electron Microscopy (figure 4). X-ray diffraction data (figure 5) suggests that these small particles likely consist of mostly clay particles, which is also what we can see in our electron microscopy photos.

Figure 2: Particle size distribution of Piedmont stormwater, determined by pumping 1ml of sample through Occhio flow-imaging particle size analyser.

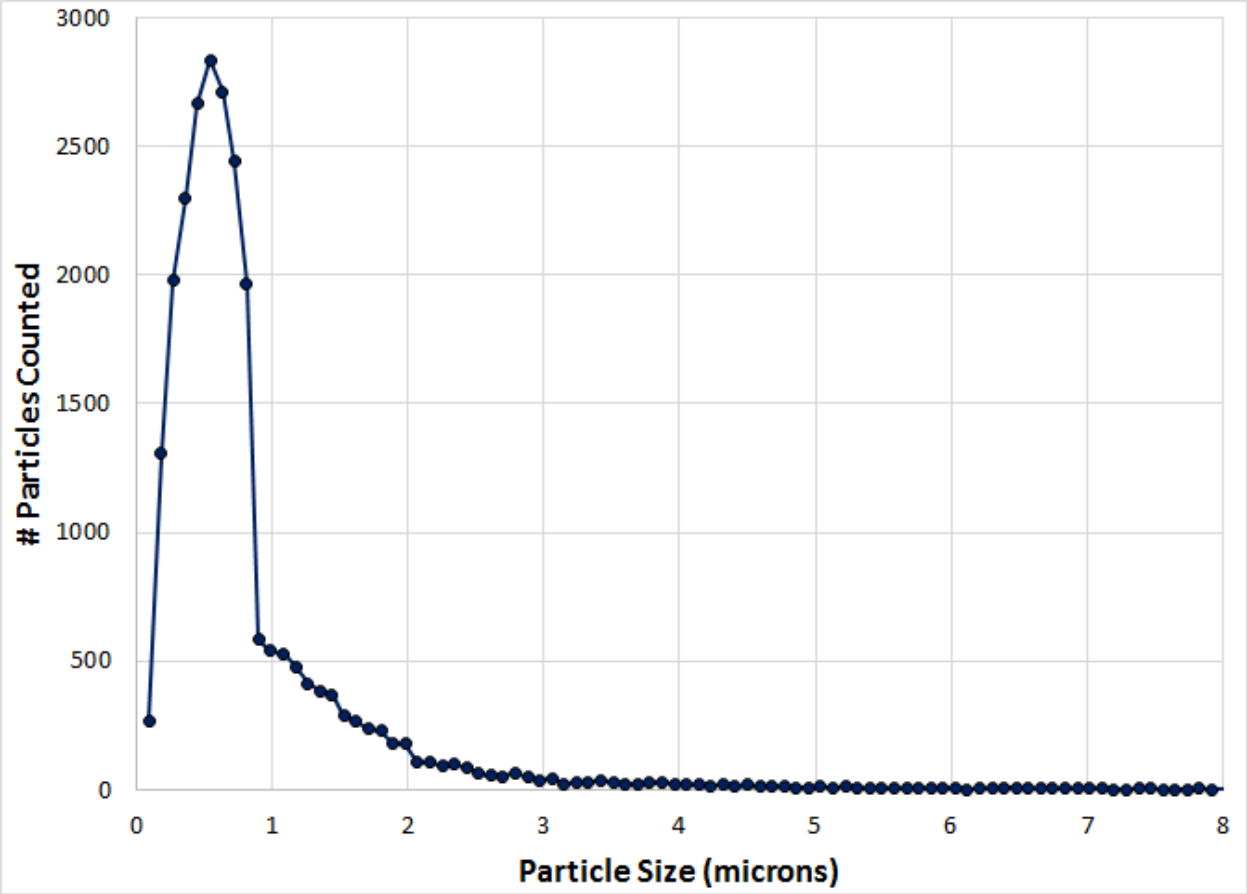


Figure 3: Screenshot of flow-imaging particle analysis of: a) 4.6μ QC particles, b) weak micro-ground coffee, c) Piedmont stormwater, d) Piedmont stormwater through 0.02μ filter.

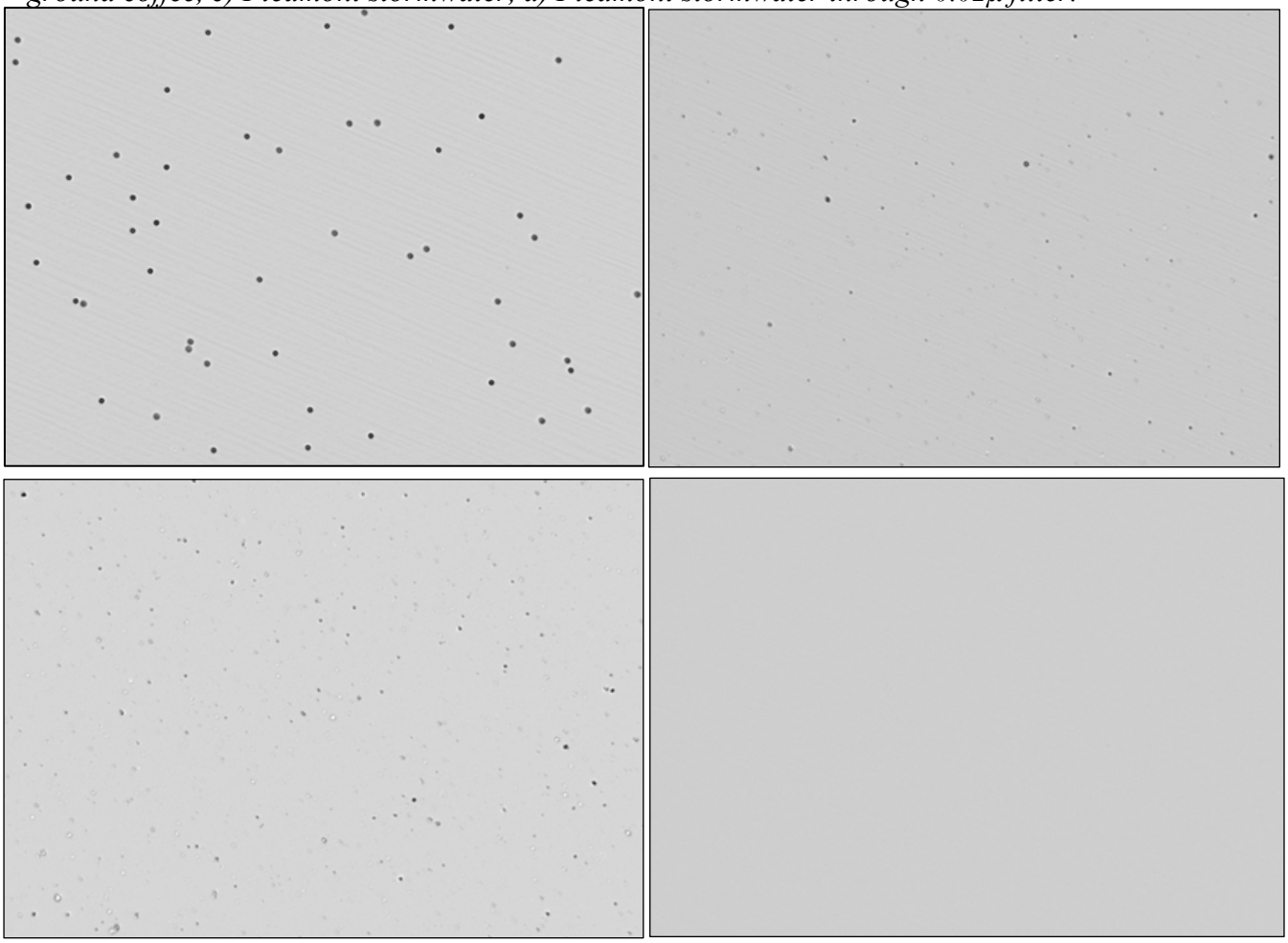


Figure 4: Typical scanning electron microscopy image of stormwater particles, used as an independent technique to verify the particle size distribution obtained via flow-imaging.

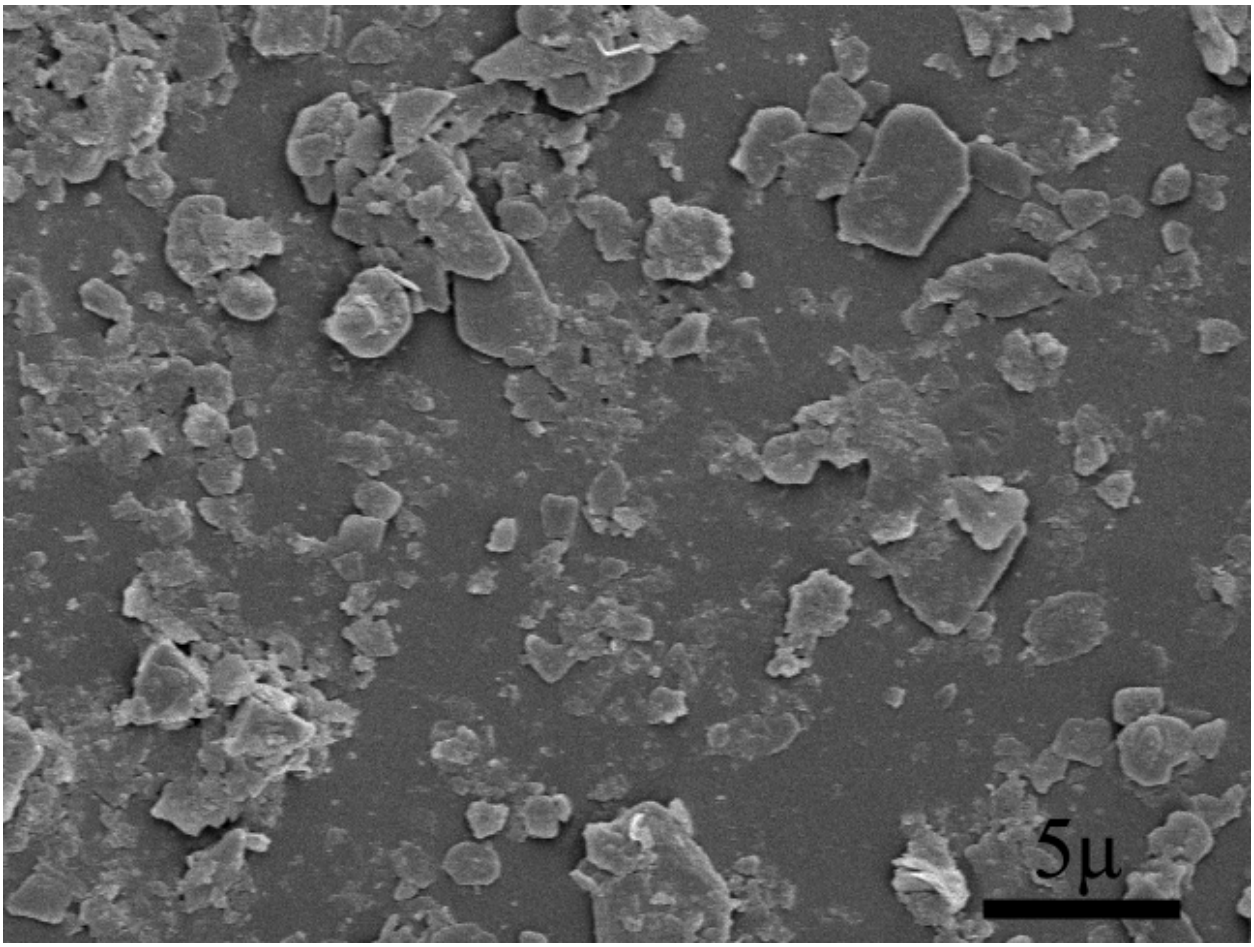
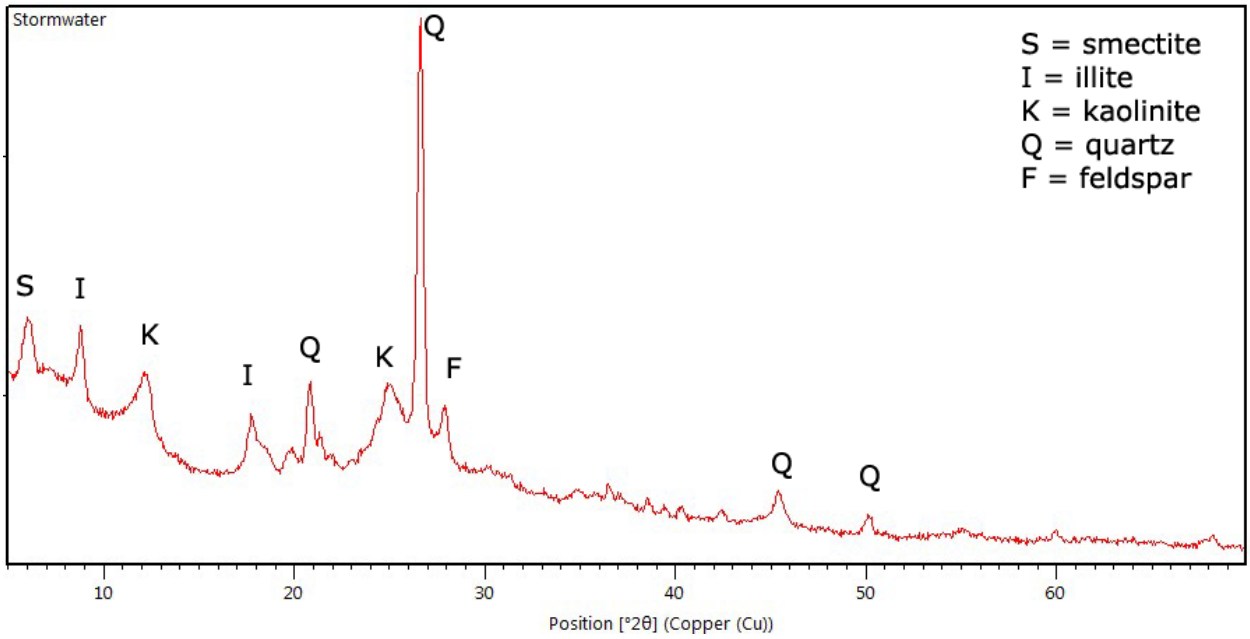


Figure 5: X-ray diffraction data from a typical stormwater sample from the North Carolina Piedmont. Predominant minerals include quartz, feldspar, smectite, illite, and kaolinite.



We are still analyzing data for phosphorus and nitrogen, and will include this data in our final report.

Significance of Findings to date:

The strong relationship between turbidity and particles holds great promise for the use of in-situ or ex-situ turbidity sensors as a tool to estimate suspended sediment concentration and flux (when paired with a USGS stream gauge).

The small particles in Piedmont stormflow means that sediment-bound contaminants are difficult to remove once these particles get suspended, since Stokes' Law predicts that these particles can take days to settle out of the water column. This has implications for stormwater best management practices (BMP's).

Appendix 1

Abbreviations:

BMP: best management practice

N: nitrogen

P: phosphorus

SEM: scanning electron microscopy

TSS: total suspended sediment

USGS: United States Geologic Survey

Appendix 2

Publications:

Particle Size Distribution Predicts Particulate Phosphorus Removal: a Mechanistic Model and Implications for Stormwater BMP's. Submitted to Ambio in March 2017 (currently under review).

Presentations:

Particulate Phosphorus in Stormwater. International Phosphorus Workshop, Rostock, Germany, September 2016.

Impact of Hospital and Patient Discharges on North Carolina Surface and Drinking Water Quality as Measured by Iodinated Contrast Agents

Basic Information

Title:	Impact of Hospital and Patient Discharges on North Carolina Surface and Drinking Water Quality as Measured by Iodinated Contrast Agents
Project Number:	2016NC200B
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Descriptors:	None
Principal Investigators:	Howard Weinberg, Kirsten E Studer

Publications

There are no publications.

Impact of Hospital and Patient Discharges on North Carolina Surface and Drinking Water Quality as Measured by Iodinated Contrast Agents

WRRI Project 16-10-W
Covering the period from 03/01/2016 to 02/28/2017

SUBMITTED ON: 05/19/2017

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Keywords: iodinated disinfection byproducts, surface water quality

1. Introduction

Due to the absence of or limited regulations on medical discharge to the sanitary sewer, many pharmaceutically active agents from hospitals and patients reach wastewater treatment plants, which may not be able to adequately remove them. Among these are iodinated-contrast agents which several large hospitals and many medical facilities across the state administer to patients for soft tissue imaging. The contrast media are applied at high doses and are eliminated through urine and feces without metabolization of the parent compound (Pérez et al. 2006). Previous studies have observed evidence that these agents break down in wastewaters, releasing iodine that could appear in surface waters (Kormos et al. 2011; Kovalova et al. 2013; Mu et al. 2011; Nelson et al. 2011; Putschew et al. 2000; Ternes & Hirsch 2000; Wendel et al. 2014). Fuge and Johnson (1986) reported that U.S. rivers and freshwater lakes show a wide range in natural iodide levels of 0.01 to 73.3 μg/L, but with no reported levels of iodide in North Carolina (NC) surface waters. One specific occurrence study of twenty-three cities observed iodinated disinfection byproducts (DBPs) in drinking waters whose sources contained low iodide concentrations that were determined to be a result of medical imaging compounds reacting with drinking water disinfectant (Richardson et al. 2008; Duirk et al. 2011). Epidemiological studies have indicated a weak correlation between chlorinated drinking water exposure and various types of human cancer, with bladder, colon, and rectal cancers being the primary disease endpoints, and the health implications focusing on DBPs (Hildesheim et al. 1998; Villanueva et al. 2007). From the known byproducts, the non-regulated iodinated DBPs have been shown to have an elevated toxicity in comparison to other DBPs (Richardson et al. 2008). According to (Plewa et al. 2004), iodinated DBPs are over 250 times more cytotoxic than the regulated chloroacetic acid. Due to toxicity of iodinated DBPs, the U.S. Environmental Protection Agency (EPA) has identified these compounds, along with brominated species, as DBPs of emerging toxicological interest (Richardson 2003).

Generally, iodinated DBPs are formed from chloramine disinfection of source drinking water containing iodide, with a shorter contact time increasing their formation (Bichsel & von Gunten 1999). With the potential for iodine in NC surface waters from hospitals and medical facilities and widespread use of chloramine for disinfection of drinking water, there is an increased risk of iodinated byproduct formation in finished drinking water and it is, therefore, prudent for additional attention to be focused on the impact of iodinated contrast agents on drinking water quality and the subsequent health risk.

Currently, the waste streams from NC hospitals and other medical facilities are not monitored or regulated even though contrast media are very persistent and conventional wastewater treatment plants do not have processes that can remove inorganic iodide or hydrophilic organic iodine (Ternes & Hirsch 2000; Hollender et al. 2009). If the impacted surface water is then used as a source for drinking water, the presence of this organic iodine can lead to formation the of iodinated DBPs, especially when chloramine disinfection is used, and potentially elevate the water's toxicity and potential health risk to consumers.

2. Significance to NC Water Resources

Since most medical waste is neither regulated or monitored in NC, the discharge from hospital waste streams has not previously been studied, yet it is important to identify potential

anthropogenic impacts to our waterways that could adversely affect the quality of our drinking water. With the use of increasingly impacted waterways as drinking water sources in NC, it is imperative to identify waste streams that are not completely mitigated from the wastewater treatment plant and whose residues, such as iodine, can act as precursors to DBPs. Selection of new drinking water sources in rapidly growing parts of the state can also be informed by this study and the impact of medical waste streams on drinking water quality.

North Carolina has little to no naturally occurring iodine levels in surface waters; however, there are several large hospitals and many facilities across the state that administer iodinated contrast agents which could introduce iodine into surface waters that are drinking water sources downstream of wastewater treatment plants. If the drinking water plants use chloramine for disinfection there is a likelihood that iodinated DBPs will be formed, among which are one class called iodoacids. If correlations can be made from iodinated-contrast media in medical waste and iodoacids in finished drinking water, then recommendations can be made for appropriate medical waste pretreatment and policy to protect drinking water plant source waters.

Since the impact of contrast agents has not been previously investigated in NC, this study has selected a watershed for a case study to determine if upstream wastewater discharges from hospitals to treatment plants are contributing to iodinated DBP formation in a downstream drinking water treatment plant which disinfects with chloramine. According to the NC Hospital Association, there are over 130 hospitals in the North Carolina and many more clinics that provide contrast-assisted imaging services (NCHA 2017). To help protect the surface water and source drinking water in North Carolina, it is imperative to access the persistence of iodinated-contrast agents in medical waste, surface water, and drinking water. Without studying the persistence of these contrast agents, regulatory agencies will lack pertinent information with which they can make accurate judgments that directly affect public health in the state.

3. Project Objectives

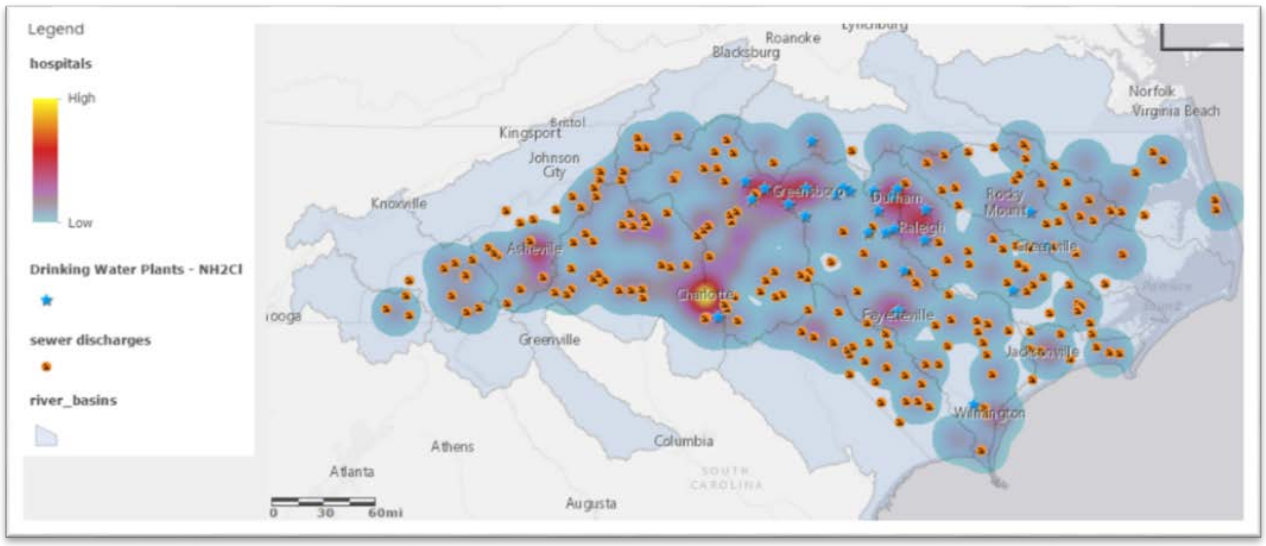
This project looked at the impact of iodinated contrast agents from hospital wastewater on surface water iodine levels in a selected NC watershed and their direct impact as precursors to iodinated DBPs. The project milestones included identifying compromised surface waters receiving medical waste, identifying drinking water treatment plants with impaired sources, and determining the drinking water quality parameters associated with iodinated DBP formation. It was hypothesized that surface waters with treated hospital waste effluent increased the levels of iodine and that drinking water plants with chloramines using these impacted surface waters would contain iodinated DBPs.

4. Identification of High Priority Sampling Areas

The locations of medical diagnostic facilities were identified using the state's records for licensing of X-ray, magnetic resonance imaging, and computed tomography (NCHA 2017). With the aid of the North Carolina Urban Water Consortium (NC UWC), drinking water treatment plants (DWTPs) using chloramination for disinfection of surface waters were also identified and each DWTP was geocoded in order to spatially determine the possible impacts of medical waste. ArcGIS Online (ESRI 2017) was used to construct maps for selecting high priority sampling areas and Figure 1 shows the regional density of hospitals in North Carolina

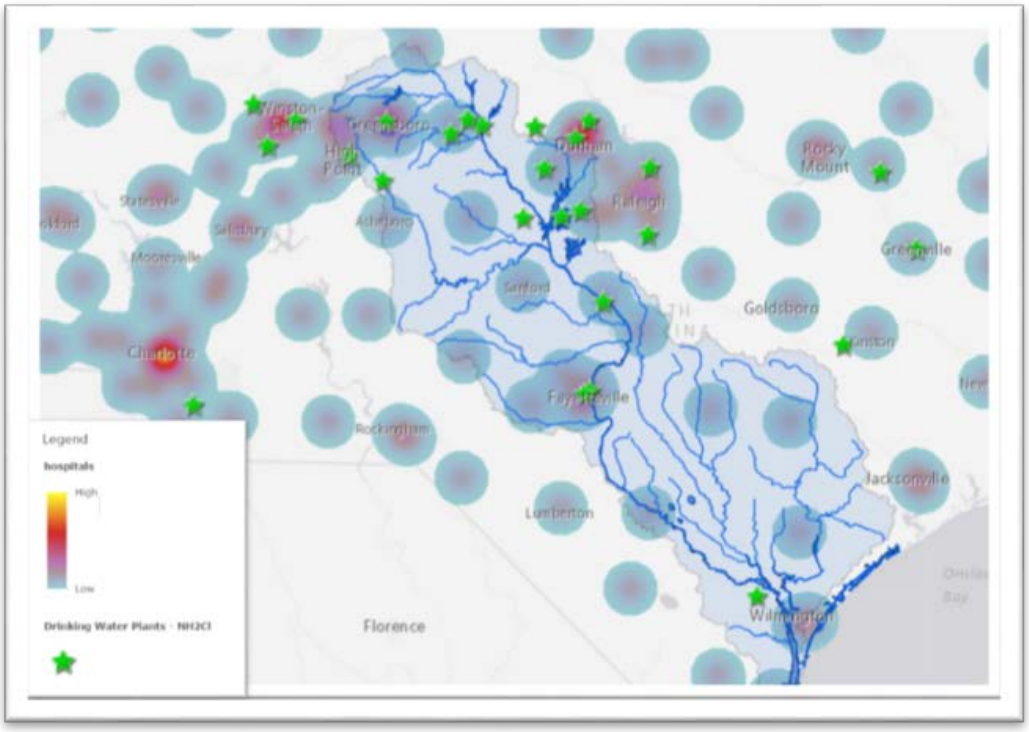
with the 27-surface water-chloraminating DWTPs in the state. Appendix 3 includes a table of the 27 treatment plants and the surface waters each utility used as a drinking water source.

Figure 1: Map of Hospital and Medical Center Density in Proximity to Drinking Water Treatment Plants Using Chloramine for Disinfection



The preliminary high priority sampling locations were selected due to the proximity of the chloraminating DWTPs to the wastewater effluent impacted by medical waste. The greatest concentration of chloraminating DWTPs was in the Cape Fear River Basin (see Figure 2).

Figure 2: Cape Fear River Basin with Locations of Drinking Water Treatment Plants Using Chloramine for Disinfection and Hotspots for Hospital Density



Based on the proximity to multiple hospitals and the University of North Carolina Chapel Hill campus, the iodine-impact analysis focused on the man-made reservoir in the Upper Cape Fear Basin, B. Everett Jordan Lake. According to the NC Surface Water Assessment Program Report (NCDEQ 2015), Jordan Lake is highly impacted by upstream point and non-point sources and serves as the primary drinking water source for a population of about 160,000.

Once the target area was identified, upstream wastewater treatment plant effluents and drinking water treatment plant source and finished water were collected to determine the levels of iodine and other water quality parameters as a preliminary screening. Finished water from drinking water treatment plants was also assessed for iodinated-DBPs to determine the impact of the iodinated contrast waste on drinking water quality.

5. Analytical Methods

The analytical methods for iodine and iodinated DBPs used a variety of techniques which measured total iodine (TI), total inorganic iodide (TI), and total organic iodine (TOI). Additional water quality parameters, including total organic carbon and total nitrogen, were determined for each surface and treated water sampled. Table 1 outlines the water quality parameters monitored.

Table 1: Parameters for Iodine Analysis in Wastewater Effluent, Surface Water, and Drinking Water

Parameter	**Instrument**	**Volume needed**
Total Iodine	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	20 mL
Inorganic Iodine	Ion Chromatography - Electroconductivity Detector (IC-ED)	20 mL
Organic Iodine	Total Organic Halide (TOX) Analyzer, ICP-MS and IC-ED	150 mL
Total Organic Carbon/ Total Nitrogen	Shimadzu TOC-V_{CPH} and TOC-V_{CPN} Analyzer	50 mL

A mass balance of iodine from total iodine measurement using inductively coupled plasma – mass spectrometry (ICP-MS) (Takaku et al. 1995) and fractionation of total organic halides (TOX) into total organic iodine (Hua & Reckhow 2006) was used to assess the impact of iodinated contrast agents on downstream drinking water quality when the total iodine load was accounted for within the engineered water cycle. Specifically for the drinking water quality assessment, the amount of unknown iodinated DBPs can be calculated from the difference in mass between TOI and the sum of the measured iodinated DBPs, specifically iodinated trihalomethanes and iodinated haloacetic acids. The methods selected for this project allow a direct assessment of the impact of iodinated contrast agents on North Carolina water quality.

6. Case Study of Jordan Lake

Sampling locations for each wastewater treatment plant impacting Jordan Lake were determined based on monitoring locations selected by the three individual wastewater utilities, with sampling points along small waterways prior to discharge and post discharge of the treated

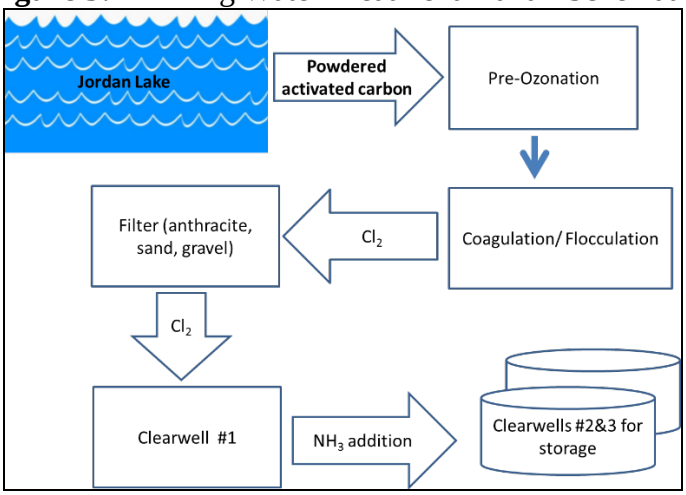
effluent (see Table 2). Sampling of the receiving streams was conducted on the same day as the wastewater collection and all grab samples were collected at each location within the watershed in a 48 hr sampling window to ensure that weather events did not impact the analysis.

Table 2: Sampling Locations for Iodine-Impact Analysis

Surface Waters Sampling	Locations	Characteristics
Morgan Creek	Pre and post wastewater treatment plant (WWTP) discharge points	• UV disinfection • WWTP 3 discharge of 6.0 MGD • Waste from large regional hospitals received at WWTP
New Hope Creek	Pre and post WWTP discharge points	• UV disinfection • WWTP 2 discharge of 9.3 MGD • Waste from large regional hospitals received at WWTP
Northeast Creek	Pre and post WWTP discharge points	• UV disinfection • WWTP 1 discharge of 5.0 MGD
Drinking water treatment plant 1 (DWTP 1)	Jordan Lake as drinking water source	Chloramination of surface water
Haw River	Upriver from joining Jordan Lake	Upper Cape Fear River Basin
Drinking water treatment plant 2 (DWTP 2)	Cape Fear River as drinking water source	Chloramination of surface water

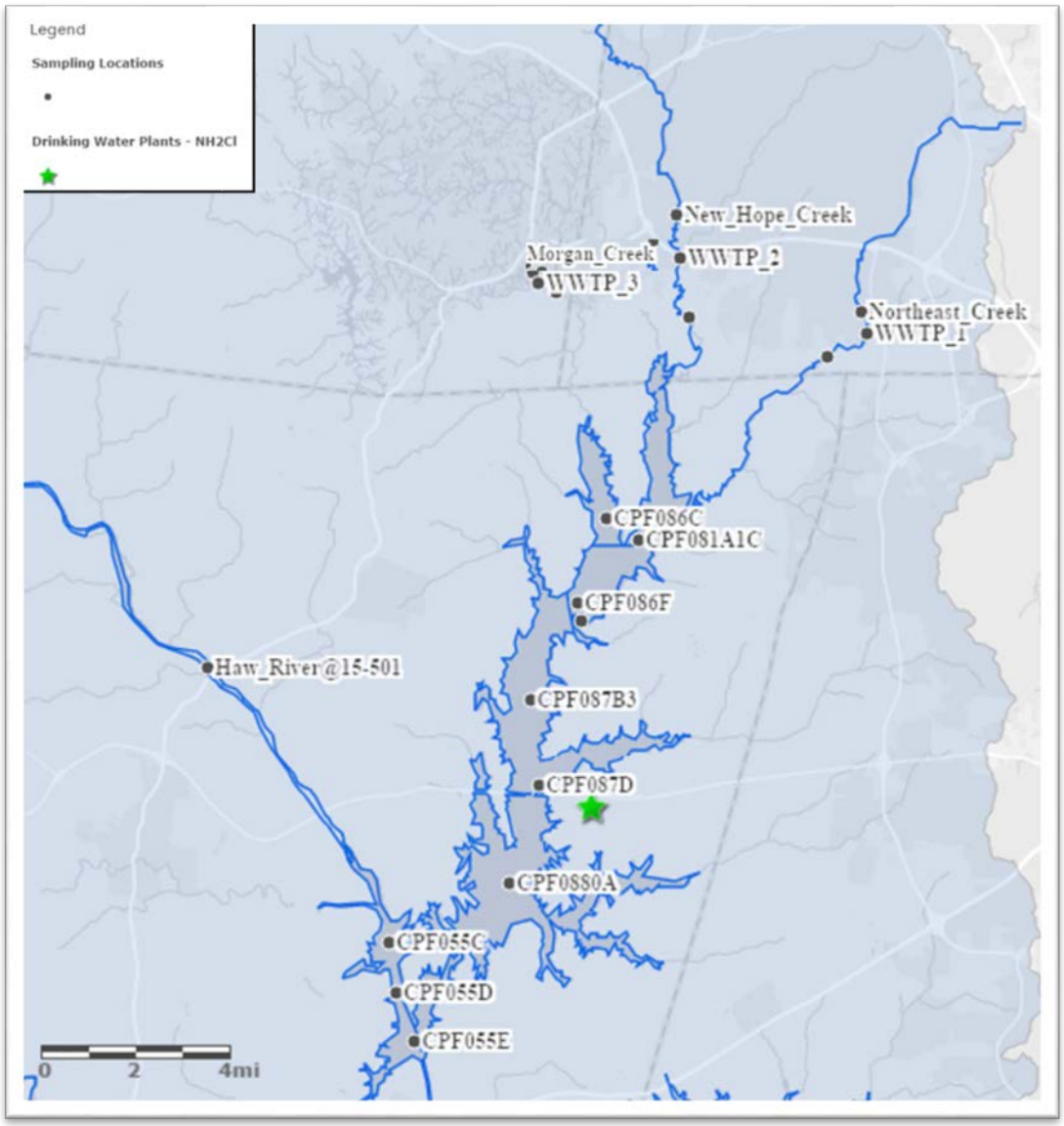
Drinking water treatment plant 1 (DWTP 1) uses Jordan Lake as its primary water source and its treatment regime is outlined in Figure 3. Samples were collected at the intake, within the plant, and the treated effluent to assess iodine impact on finished water quality.

Figure 3: Drinking Water Treatment Plant 1 Schematic



Additionally, staff from the NC Department of Environmental Quality, Division of Water Resources collected samples from 9 monitoring stations on Jordan Lake. Figure 4 shows each sampling location within the Cape Fear River Basin, except DWTP 2, and the Jordan Lake samples are indicated by the codes beginning with Cape Fear (CPF).

Figure 4: Wastewater Treatment Plants and NC Division of Water Resources Sampling Locations for the Jordan Lake Watershed



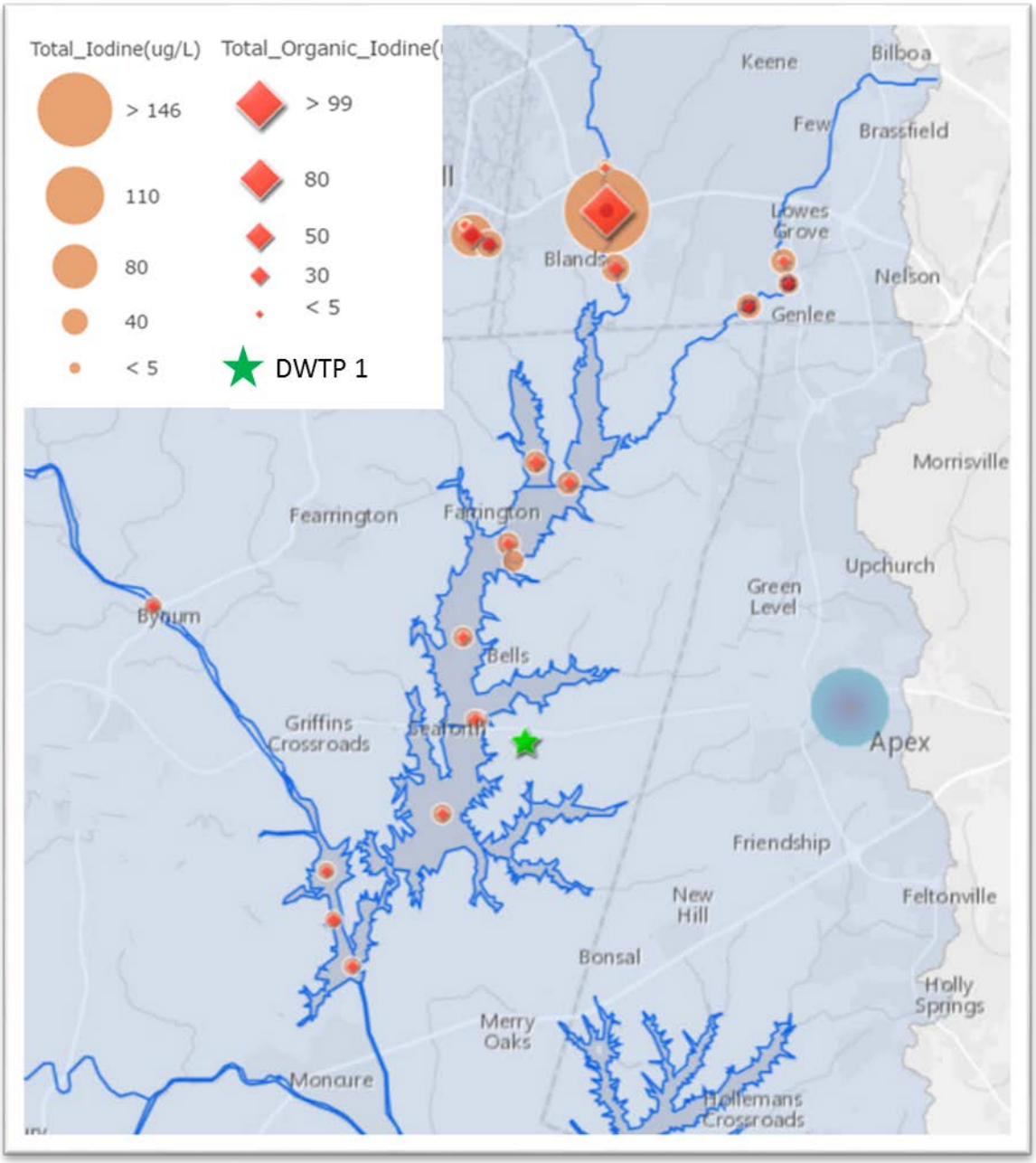
7. Preliminary Results

Samples collected in a first survey of Jordan Lake showed sources of organic iodine entering the lake and reaching the intake of a downstream drinking water treatment plant, located on Figure 4 as the star, that uses the surface water as its primary water source. The preliminary chemical assessment shows that the bulk of the organic iodine contribution originates from two of the three wastewater treatment plants located on streams that feed the upper arms of Jordan Lake and which are known to treat wastewater from large hospitals. Table 3 shows a detailed overview of the water quality assessment for each sampling location. Figure 5 provides a spatial distribution of the iodine assessment, which highlights the input of the wastewater treatment plants on Morgan Creek and New Hope Creek to Jordan Lake.

Table 3: Water Quality Data for Each Monitoring Location Collected on 2/14/17 and 2/15/15

Sampling Location	**Total Iodine** µg/L as I	**Total Organic Iodine** µg/L as I	**Total Organic Carbon** mg/L as C	**Total Nitrogen** mg/L as N	**Dissolved Organic Carbon** mg/L as C	**Dissolved Nitrogen** mg/L as N
Northeast Creek Pre-WWTP	30.3	12.5	6.20	0.27	6.28	0.35
WWTP 1	22.4	11.7	5.23	4.14	5.27	4.49
Northeast Creek Post-WWTP	29.3	13.3	6.27	2.98	5.43	3.25
New Hope Creek Pre-WWTP	14.1	6.97	4.88	0.20	4.99	0.29
WWTP 2	146.8	99.3	7.23	5.70	6.65	5.86
New Hope Creek Post-WWTP	36.5	18.9	5.97	1.21	5.83	1.21
Morgan Creek Pre-WWTP	8.69	5.59	3.28	0.46	3.1	0.41
WWTP 3	63.2	39.1	5.03	4.98	5.00	5.18
Morgan Creek Post-WWTP	34.4	20.0	4.02	3.11	4.07	3.21
Jordan Lake CPF086C	25.6	18.9	5.52	0.57	5.39	0.54
Jordan Lake CPF081A1C	26.7	14.5	5.78	0.64	5.84	0.62
Jordan Lake CPF086F	25.7	14.3	5.68	0.69	5.70	0.58
Jordan Lake CPF087B3	21.4	12.5	5.64	0.61	5.56	0.51
DWTP 1 Intake	18.4	11.1	5.43	0.53	5.08	0.47
Jordan Lake CPF087D	19.9	14.9	5.52	0.62	5.62	0.60
Jordan Lake CPF0880A	19.6	12.5	5.29	0.61	5.51	0.64
Haw River	16.4	9.40	4.23	1.36	4.37	1.51
Jordan Lake CPF055C	15.5	8.70	4.64	1.04	4.74	1.14
Jordan Lake CPF055D	14.8	14.6	4.68	0.98	4.88	1.01
Jordan Lake CPF055E	16.2	10.9	4.81	0.82	5.05	0.91
DWTP 2 Intake	11.4	5.01	4.83	0.79	4.68	0.79

Figure 5: Total Iodine and Total Organic Iodine Analysis for Jordan Lake



Using the same water quality data as in Table 3, a correlation matrix was built to determine if trends existed within the chemical analysis. Figure 6 shows each parameter of water quality data graphed against the other data, so that linear relationships could be observed if they were present. To read this matrix, the first row has a y-axis of total iodine (TI) and the first column has a x-axis of total iodine (TI). The strong linear correlations that are observed relate to established chemical relationships, such as dissolved organic carbon (DOC) with total organic carbon (TOC) for a Pearson's correlation coefficient (r) value of 0.953. Table 4 displays the Pearson's correlation coefficient for each of the plots within the correlation matrix.

Figure 6: Graphical Correlation Matrix of Water Quality Parameters from Table 3

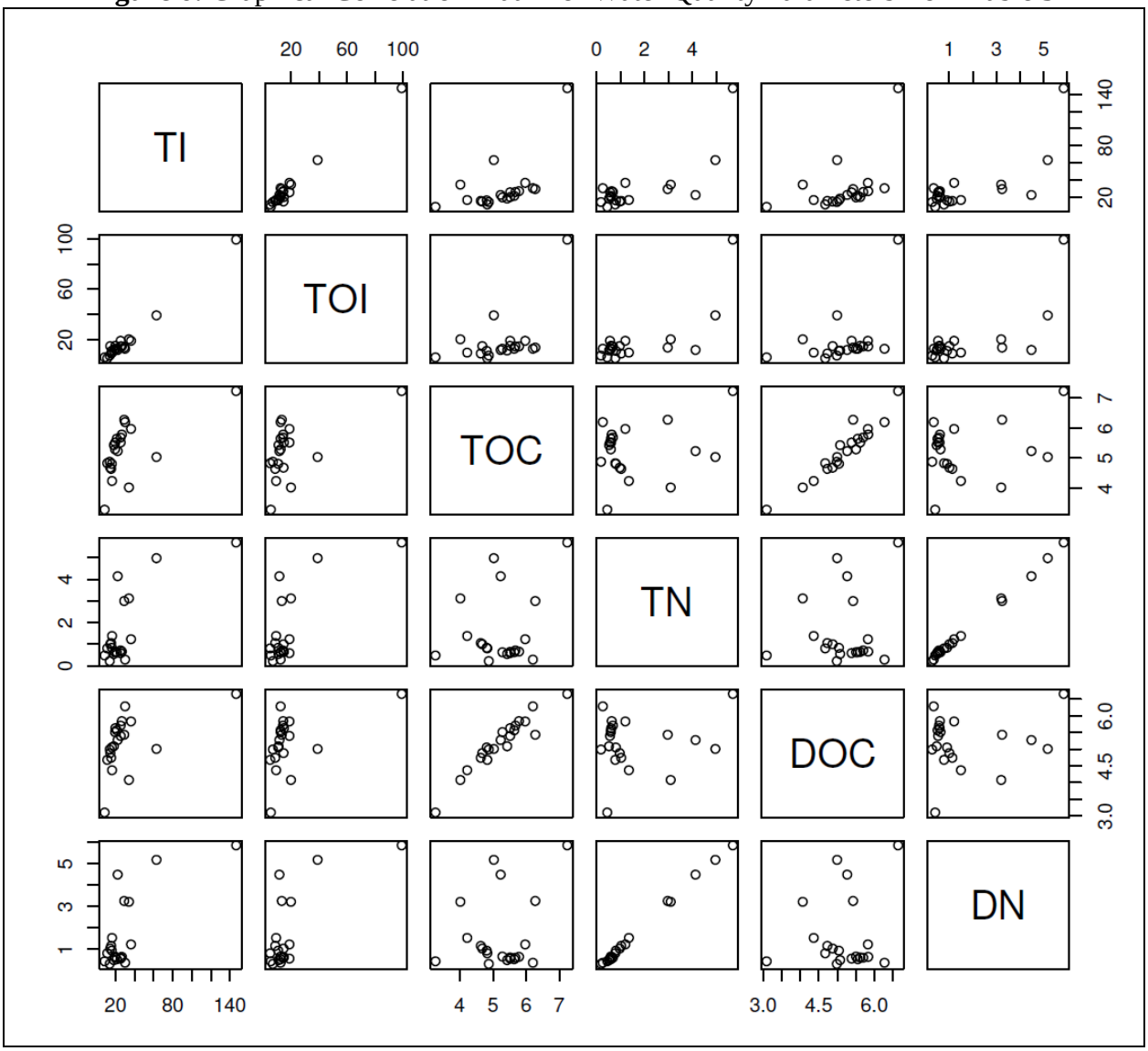


Table 4: Pearson's Correlation Coefficients (r) for Jordan Lake Water Quality Parameters

	TI	**TOI**	**TOC**	**TN**	**DOC**	**DN**
TI	--					
TOI	0.990	--				
TOC	0.599	0.554	--			
TN	0.755	0.731	0.273	--		
DOC	0.508	0.471	0.953	0.161	--	
DN	0.737	0.711	0.265	0.999	0.155	--

TOC, TN, DOC, and DN showed positive correlations when compared to TOI (r ranging from 0.471 to 0.731) as well as TI (r ranging from 0.508 to 0.755). The positive correlations between total organic iodine to total nitrogen (r=0.731) and between total organic iodine to total organic carbon (r=0.554) may suggest that the total nitrogen input could be used as an indicator for

organic iodine since they may originate from the same wastewater effluent. For the DBP analysis of the two drinking water treatment plants, additional analysis was conducted for total organic halogens (TOX), which would include total organic iodine, chlorine, and bromine. For the iodinated DBPs specifically, the TOI was quantified using ICP-MS analysis. Table 5 shows the TOX for five sampling points within DWTP 1 on Jordan Lake and two sampling points within DWTP 2 on the Haw River, approximately 50 miles downstream from Jordan Lake. The difference between the influent TOI for DWTP 1 and DWTP 2 is due to dilution effects from surface water inputs after the water leaves Jordan Lake. Another preliminary observation within DWTP 1 shows a significant reduction for TOI between the water entering the plant and before it is filtered. Based on the DWTP 1 schematic in Figure 3, this reduction in organic iodine could be a result of adsorption onto the powdered activated carbon (PAC) then removal through sedimentation or it could be from the ozonation of the organic iodine to form iodate, an inorganic form of iodine. This removal or transformation of organic iodine will be further investigated through iodate analysis of the DWTP 1 pre-filter samples.

Table 5: DBP Analysis in terms of Total Organic Halogens and Total Organic Iodine

Samples	Total Organic Halogen (µg/L as Cl)	Total Organic Iodine (µg/L as I)
DWTP - 1 Influent	83	11.1
DWTP - 1 Pre-Filter	114	0.5
DWTP - 1 Post-Filter	113	1.4
DWTP - 1 Clearwell 1	127	1.3
DWTP - 1 Effluent	194	2.0
DWTP - 2 Influent	134	5.0
DWTP - 2 Effluent	156	2.0

8. Continuing Work

The next steps in this continuing project include continuing to monitor organic iodine inputs to surface waters in the selected watershed used for drinking water and confirming source of organic iodine using high-resolution mass spectrometry for chemical identification. Further speciation of organic iodine using non-target analysis can permit an evaluation of iodine that supports additional mass balance of iodine. The concentration of inorganic iodine (iodate and iodide) for each sampling location will also be measured to determine the iodine mass balance.

To further explore the correlation between TOI and organic matter (OM) in the water samples, excitation-emissions matrix (EEM) fluorescence spectroscopy will be used to determine the chemical characteristics of the organic carbon and organic nitrogen present in wastewater and surface water. EEM fluorescence spectroscopy allows for the characterization of OM using the ratio of emission to excitation intensity of known fluorophores at specific wavelengths for the hydrophobic acid fraction (Peak A, $\lambda ex/\lambda em \sim 260/380–420nm$), humic-like fraction (Peak C, $\lambda ex/\lambda em \sim 350/420– 480nm$), and hydrophobic base (or protein-like) fraction (Peak T, $\lambda ex/\lambda em \sim 220/303$ nm) (Mcknight et al. 2001; Stedmon et al. 2003; Murphy et al. 2008). Additionally, the ratio of fluorescence intensities for Peak T to Peak C can indicate the biochemical oxygen demand relative to the dissolved organic carbon of the water, which can be

used to determine the impact of wastewater on a water body (Gabor et al. 2014; Ma et al. 2001; Hudson et al. 2007).

The treated water samples will also be used for the iodoacid analysis, specifically iodoacetic acid, chloroiodoacetic acid, bromoiodoacetic acid, and diiodoacetic acid that have been shown to have higher toxicity levels relative to currently regulated DBPs (Plewa et al. 2004). Iodoacids can be found in the same extract as other haloacetic acids but they are orders of magnitude smaller in abundance and detection sensitivity. New methods have, therefore, been developed to increase the extraction concentration factor and detection limit of iodoacids. (Weinberg et al. 2011) developed a multiple step extraction method for iodoacids from drinking water using a higher concentration factor without increasing the signal interference or baseline noise. This method uses larger sample volumes for liquid-liquid extraction, followed by solid phase extraction, and a second liquid-liquid extraction prior to derivatization and instrumental analysis.

9. Student Involvement

This award has been used to fund the doctoral research of one PhD student. As a direct result of this award, we collaborated with two NC drinking water treatment plants, three NC wastewater treatment plants, and the NC Department of Environmental Quality Division of Water Resources for the collection of samples throughout the state, specifically in the Cape Fear River Basin. This project has been given a no-cost extension until August 2017 to continue the research on drinking water quality in North Carolina. At the conclusion of this project, we plan to provide water quality information gained throughout this study to aid utilities in better understanding wastewater impacts to source drinking water. One rising second year undergraduate at UNC will be joining the project this summer for her research experience and will be primarily mentored by the doctoral student.

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Appendix 1: Abbreviations and Symbols

Cl – chlorine
CPF – Cape Fear
DBP – disinfection byproducts
DN – dissolved nitrogen
DOC – dissolved organic carbon
DWTP – drinking water treatment plant
ECD – electron capture detector
EEM – excitation emissions matrix
ESRI - Environmental Systems Research Institute
FEST – Facilities, Engagement, Science, and Training
GC – gas chromatography
GIS – geographical information systems
I – iodine
IC – ion chromatography
ICP – inductively coupled plasma
MGD – million gallons per day
MS – mass spectrometry
m/z – mass per charge
NC – North Carolina
NCHA – North Carolina Hospital Association
NIEHS – National Institute of Environmental Health Sciences
OM – organic matter
PAC – powdered activated carbon
TI – total iodine
TN – total nitrogen
TOC – total organic carbon
TOI – total organic iodine
TOX – total organic halogens
UV_{254} – ultraviolet absorbance at 254 nm
UWC – Urban Water Consortium
WASA – Water and Sewer Authority
WWTP – wastewater treatment plant

Appendix 2: Presentations Associated with Award

Kirsten E. Studer and Howard S. Weinberg "Impact of Hospital Waste on Drinking Water Quality: Disinfection Byproduct Formation Implications from Anthropogenic Contributions" *National Institute of Environmental Health Sciences (NIEHS) Environmental Health Sciences: Facilities, Engagement, Science, and Training (FEST)* (December 2016). Durham, NC. Poster Presentation.

Kirsten E. Studer and Howard S. Weinberg "Impact of Hospital and Patient Discharges on North Carolina Surface and Drinking Water Quality as Measured by Iodinated Contrast Agents." *NC Water Resources Research Institute Conference* (March 2017). Raleigh, NC. Oral Presentation.

Appendix 3: Locations of Drinking Water Treatment Plants Using Chloramines on Surface Water in North Carolina

Drinking Water Treatment Plants	Surface Water Source	City	Zipcode
E.M. Johnson DWTP	Falls Lake Reservoir	Raleigh	27614
Dempsey E. Benton DWTP	Lake Benson and Lake Wheeler	Garner	27529
Town of Cary/Apex DWTP	Jordan Lake	Apex	27523
Orange Water and Sewer Authority	University Lake and Cane Creek Reservoirs	Carrboro	27510
Town of Hillsborough DWTP	Eno River	Hillsborough	27278
Williams DWTP	Lake Michie and Little River Reservoir	Durham	27705
Brown DWTP	Lake Michie and Little River Reservoir	Durham	27712
Chatham County DWTP	Jordan Lake	Apex	27502
Town of Pittsboro DWTP	Haw River	Pittsboro	27312
Mitchell DWTP	Lake Brandt	Greensboro	27408
Townsend DWTP	Lake Townsend	Graham	27253
Robert A. Harris DWTP	Dan River	Eden	27288
Piedmont Triad Water Authority	Randleman Regional Reservoir	Randleman	27317
Ward DWTP	Oak Hollow and City Lake	High Point	27260
JD Mackintosh DWTP	Great Alamance Creek	Burlington	27215
Ed Thomas DWTP	Stoney Creek Reservoir, Lake Cammack, Lake Mackintosh	Burlington	27217
Harnett Co. Regional DWTP	Cape Fear River	Lillington	27546
P.O. Hoffer DWTP	Cape Fear River	Fayetteville	28301
Glenville Lake DWTP	Glenville Lake	Fayetteville	28301
P. W. Swann DWTP	Yadkin River	Pfafftown	27040
R. W. Neilson DWTP	Yadkin River	Clemmons	27012
Thomas DWTP	Yadkin River, Salem Lake	Winston-Salem	27107
Greenville Utilities DWTP	Tar River	Greenville	27834
Neuse Regional WASA	Black Creek, Upper Cape Fear Aquifer	LaGrange	28551
Northwest DWTP	Cape Fear River	Leland	28451
Town of Tarboro DWTP	Tar River	Tarboro	27886
Catawba River DWTP	Catawba River	Indian Trail	28079

Comparing the Impact of Organic vs. Inorganic Nitrogen Loading to the Neuse Estuary with a Mechanistic Eutrophication Model

Basic Information

Title:	Comparing the Impact of Organic vs. Inorganic Nitrogen Loading to the Neuse Estuary with a Mechanistic Eutrophication Model
Project Number:	2016NC201B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NC-012
Research Category:	Water Quality
Focus Category:	Models, Nutrients, Water Quality
Descriptors:	None
Principal Investigators:	James Douglas Bowen

Publications

There are no publications.

Progress Report

March 1, 2016 – February 28, 2017

Comparing the Impact of Organic vs. Inorganic Nitrogen Loading to the Neuse Estuary with a Mechanistic Eutrophication Model

James D. Bowen, Principal Investigator

Department of Civil and Environmental Engineering
UNC Charlotte
Charlotte, NC 28223

WRRI Project No. 16-02-W

April 30, 2017

1. Introduction

This report summarizes the activities and findings for the WRRI Project entitled "Comparing the Impact of Organic vs. Inorganic Nitrogen Loading to the Neuse Estuary with a Mechanistic Eutrophication Model." The overall objective of the project is to update an existing two-dimensional laterally-averaged mechanistic model (Bowen and Hieronymus 2000, Bowen and Hieronymus 2003) of the Neuse River Estuary, and to use the updated model to investigate how changes in the quality and quantity of nitrogen loading to the estuary have affected water quality conditions. Several updates to the model that were completed this past year. The following updates are described in this report:

- Changes to the model grid, including relocating the downstream boundary closer to the mouth of river with the Pamlico Sound, and adding additional lateral branches to simulate water quality conditions in several large lateral creeks in the estuary,
- Extending the model time period, from the original four-year time period (June 1, 1997-December 31, 2000) to a thirteen-year time period (June 1, 1997 – May 10, 2009)
- Updating the model to the most recent version of CE-QUAL-W2 to take advantage of improvements that have been made over the past decade in various aspects of the model code, including the turbulence submodel portion of the circulation code.

Answers to two key questions will be sought once the model has been updated and calibrated:

1. How well does updated model simulate water quality dynamics?
2. What are the water quality consequences in the estuary of recent observed changes in nitrogen load quantity and quality?

The existing Neuse Estuary Eutrophication Model (NEEM) that is used as the basis of this study is an instance of the mechanistic water quality model CE-QUAL-W2 (Cole and Buchak 1995). The NEEM was developed in the early 2000's as part of the North Carolina nutrient TMDL process (Bowen and Hieronymus 2003, Stow, Roessler et al. 2003). That model schematized the river by dividing it longitudinally into sixty-one segments (Figure 1) from Streets Ferry Bridge to Oriental. Each longitudinal segment was divided into a variable number of 0.5-m thick vertical layers to simulate the variable water depths within the estuary (Figure 2). For purposes of model analysis, the model region was subdivided into five zones (river, upper estuary, middle estuary, bend, and lower estuary (Figure 2).

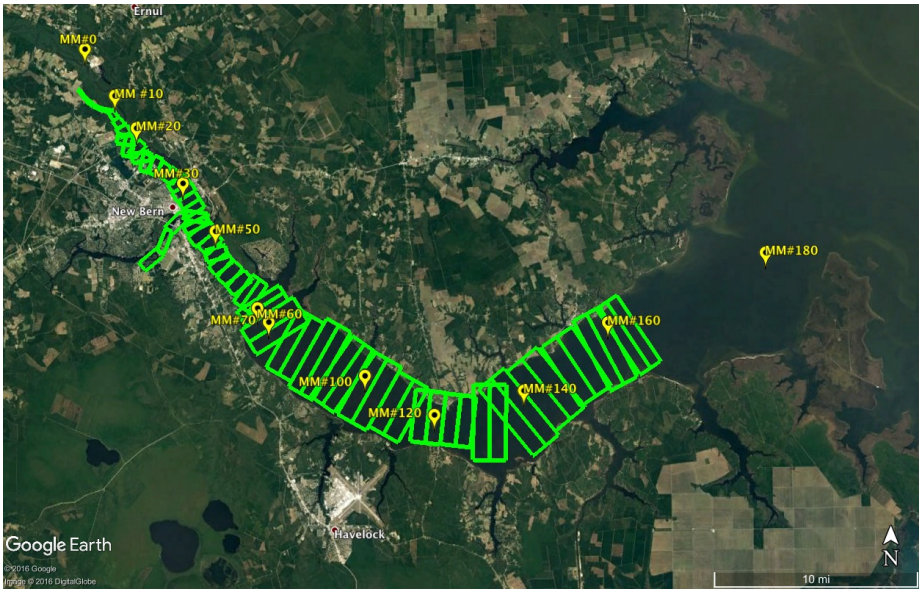

Figure 1. *61 Longitudinal segments in the original Neuse Estuary Eutrophication Model*

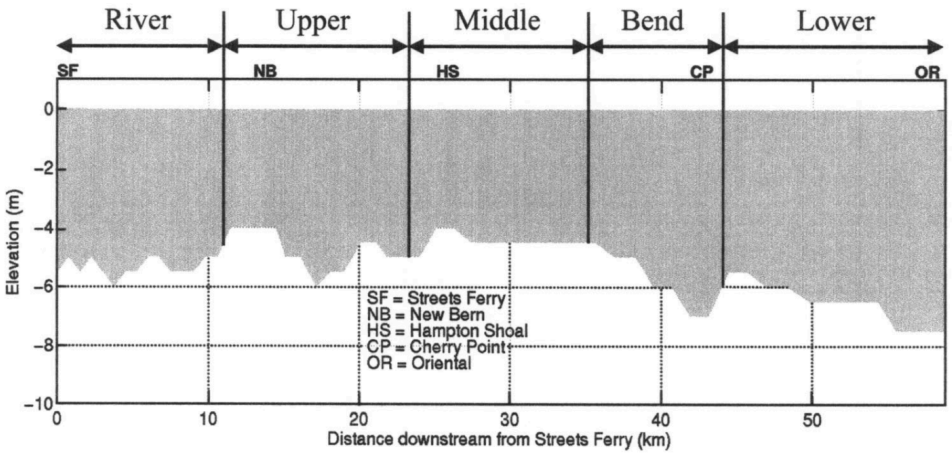

Figure 2*. Longitudinal slice of the original NEEM grid showing the variable number of layers in each segment. The horizontal axis give the longitudinal distance downstream to each model segment. The modeled region is divided into five zones from upstream to downstream.*

That original model grid had only two model branches (Neuse River, Trent River). All the remaining lateral creeks in the estuary were modeled as tributaries, meaning that the inflows from those creeks were considered to be distributed lateral inflows to designated segments along the main branch (Neuse River) of the model region. Water quality conditions in these tidal creeks were not simulated.

In the following sections of the progress report each of the bulleted model update tasks is described in more detail. The progress report concludes with a section describing the ongoing project work.

2. Model Updates

2.1 Updates to the Model Grid

The initial phase of the project concentrated on making improvements to the original model grid. To improve the model's ability to simulate nutrient load reduction scenarios it was decided to move the downstream boundary of the model to the Neuse River junction with the Pamlico Sound (Figure 3). The original model grid (Figures 1 and 2) used a downstream boundary near Oriental in part so that the nearby monitoring station (ModMon station 160, shown as MM#160 in Figure 3) could be used as the data source for the downstream water quality concentration condition. As the Modmon program developed in the early 2000's a station closer to the mouth of the Pamlico Sound (Modmon station 180, shown as MM#180 in Figure 3) was added, which enabled the relocation of the model's downstream boundary. This relocation had the advantage of expanding the modeled region and simplifying the specification of the downstream concentration condition for nutrient reduction scenarios.

At the same time the change was made in the downstream boundary, additional model segments were added for nine tidal creeks in the estuary. Four of these creeks (Goose Creek, Upper Broad Creek, Dawson Creek, Greens Creek) are located to the north of the Neuse River, while the other five creeks (Slocum Creek, Hancock Creek, Clubfoot Creek, Adams Creek, South River) are on southerly (right hand looking downstream) side of the main stem of the Neuse River Estuary. The new model grid now has a total of 150 segments, which is approximately 2.5 times as many as the 61-segment grid used for the earlier work. Despite the larger number of segments, improvements in computational speed of workstation computers produce model run times that are still significantly faster than that for the original modeling work done in the early 2000's.

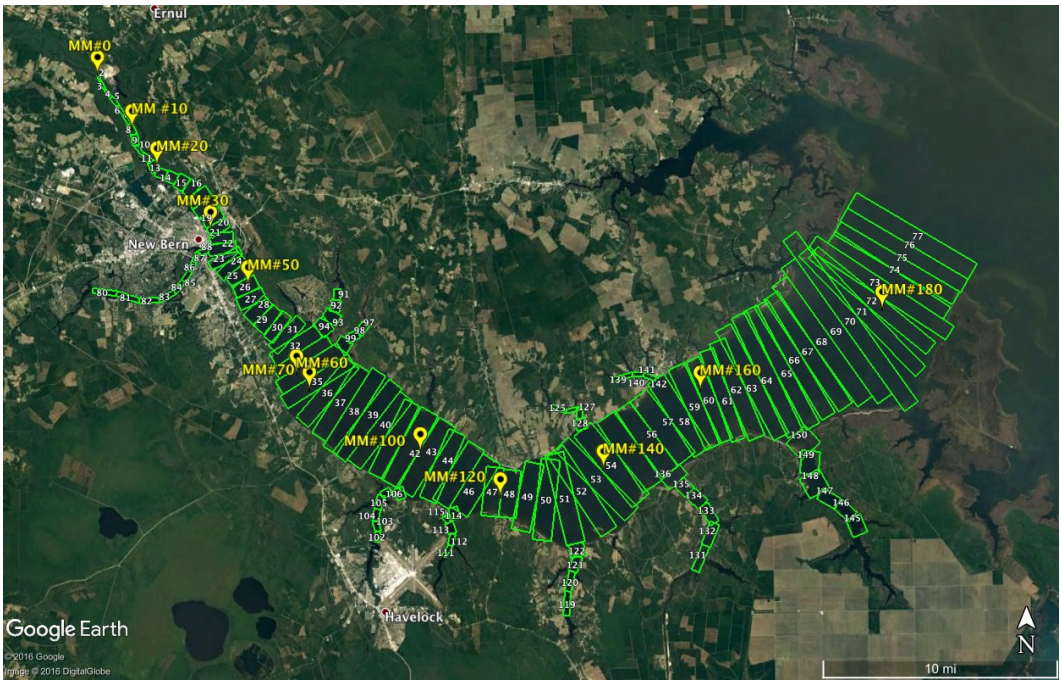

Figure 3. *Updated model grid showing relocation of the downstream boundary to the Pamlico Sound and inclusion of nine additional estuary branches*

2.2 Extension of the Model Time Period

A significant amount of project work has also been conducted this past year to take advantage of the much larger database of water quality and flow data that are now available for the Neuse River Estuary. The original TMDL work using the NEEM relied on a 42-month time period that extended from June 1997 to December 2000 (Bowen and Hieronymus 2003). Since that time, the ModMon monitoring program has continued to collect water quality and phytoplankton biomass data throughout the estuary at a monthly or semi-monthly frequency. These data have been used in a variety of ways, including an analysis of storm-event effects on nutrient-phytoplankton interactions (Paerl, Valdes et al. 2006), an examination of the effects of longer term hydrological variability on nutrients and phytoplankton biomass (Paerl, Hall et al. 2014), and as a source of data to support mechanistic and empirical models of fate and transport of free-living pathogenic bacteria (Froelich, Bowen et al. 2013).

As part of this project's work this past year, a complete model data set for running the NEEM has now been assembled using the ModMon data along with additional watershed discharge and estuarine water level data sets from the USGS, meteorological data from the National Weather Service, and riverine water quality and wastewater treatment plant discharge data from the North Carolina Division of Water Resources. The model time period has been extended by nearly nine years from the earlier model data set. The model time period now extends to June 2009, the last date for which there is monitored water level data for the estuary. It would be possible to extend the model time period even further by using simulated water level data for the estuary, which could be produced from circulation model runs such as ADCIRC (Luettich Jr, Westerink et al. 1992). We have assembled all the model data sets needed to run the model to 2014 aside from the needed water elevation data. Even without the most recent data, however, the model time period now extends for more than thirteen years and include numerous extreme weather events and exceedences of the 40 μg/L water quality criteria for chorophyll-a (Figure 4).

2.3 Update to the Current Version of CE-QUAL-W2

Once the new model grid was created, and the model data set was extended, the data set was used to create an implementation of the latest version of the two-dimensional laterally-averaged water quality model CE-QUAL-W2. The most recent version of the model (3.72) Fortran source code was released in 2015 (Cole 2015). This version of the software was compiled using the Mac and Linux versions of Intel Fortran and run on Mac workstations. Model testing has consisted of comparing the model's water quality predictions to that produced from the previous work (Hieronymus and Bowen 2004). Qualitative comparisons of time histories at two particular stations in the middle estuary (e.g. Figures 5 and 6) show very similar results between the two models.

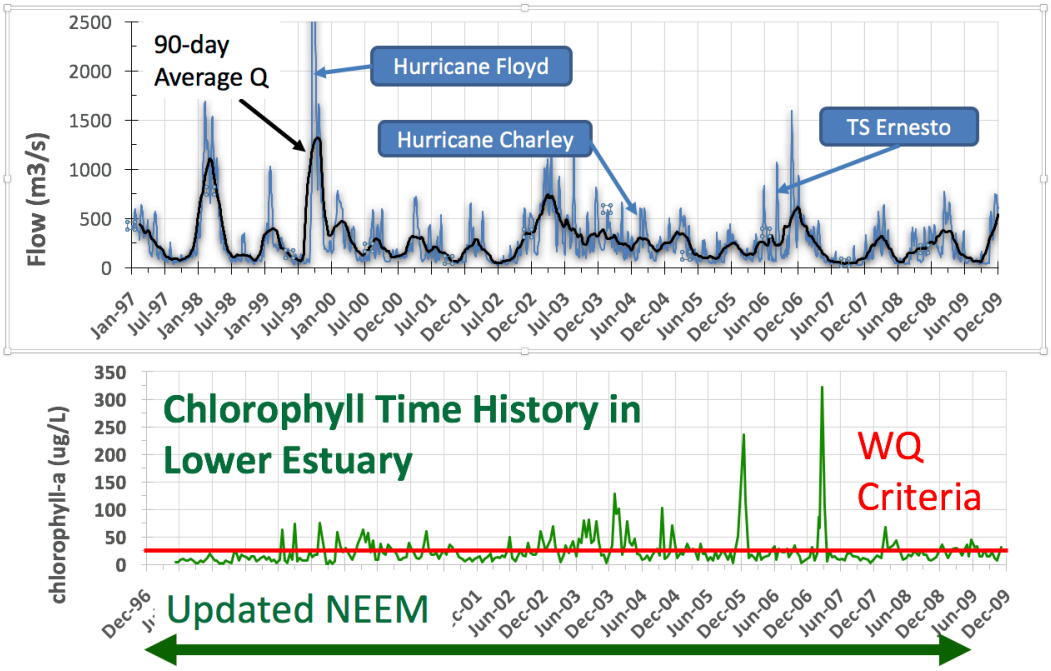

Figure 4. *Inflows to the Estuary from the Neuse River watershed (upper panel) and the observed chorophyll-a concentrations in the lower estuary (bottom panel) over the expanded thirteen-year time period of the updated Neuse Estuary Eutrophication Model.*

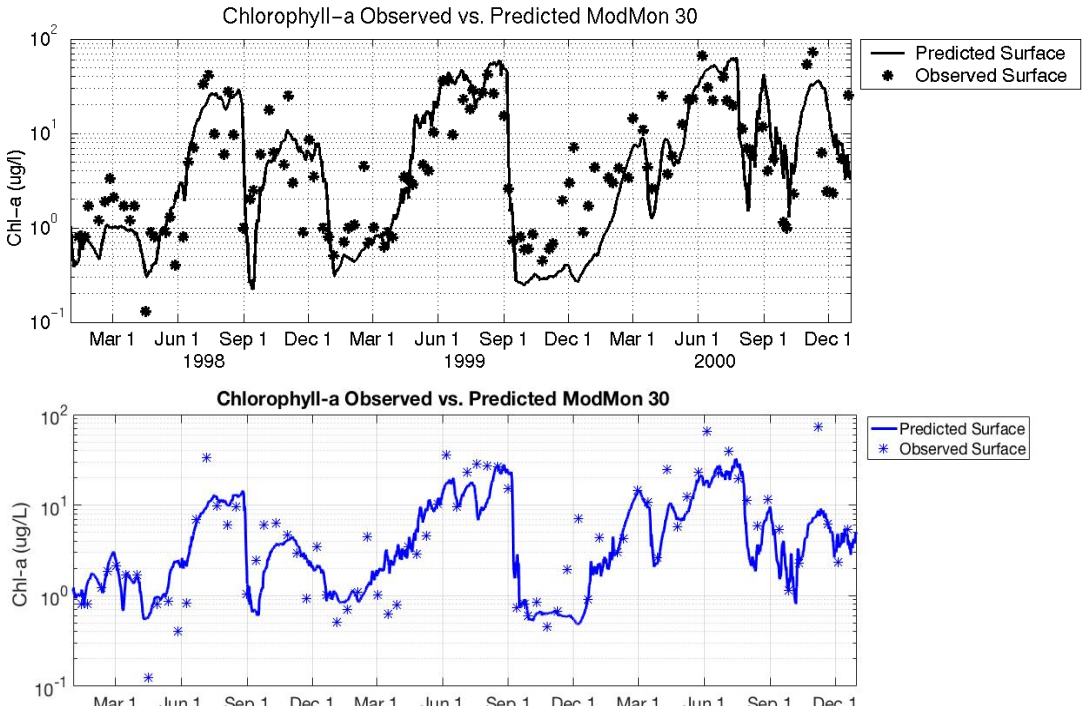

Figure 5. *Neuse Estuary Eutrophication Model predictions (solid line) and observed data (symbols) for surface conditions at ModMon station 30 for the original model (upper panel) and the updated model (lower panel).*

Some variation is expected between the original and updated models because of the many changes in CE-QUAL-W2's hydrodynamic and water quality model routines over the intervening fifteen years between the two model projects. Differences exist both in the model's computer code and in the data sets that are used to run the model. One such difference in the code is the updated vertical mixing scheme that exists in the hydrodynamic portion of the current model. In addition to code changes made by the model developer, the earlier model included several special modifications that were made specifically for the Neuse River application of CE-QUAL-W2. Two such modification are the sediment and water clarity submodels (Hieronymus and Bowen 2004) that have not yet been implemented in the new model in exactly the way they were implemented in the old model. In addition, the model's downstream boundary has been relocated and we have implemented a salinity data assimilation scheme to improve the calibration performance of the circulation model. There was no such data assimilation done previously.

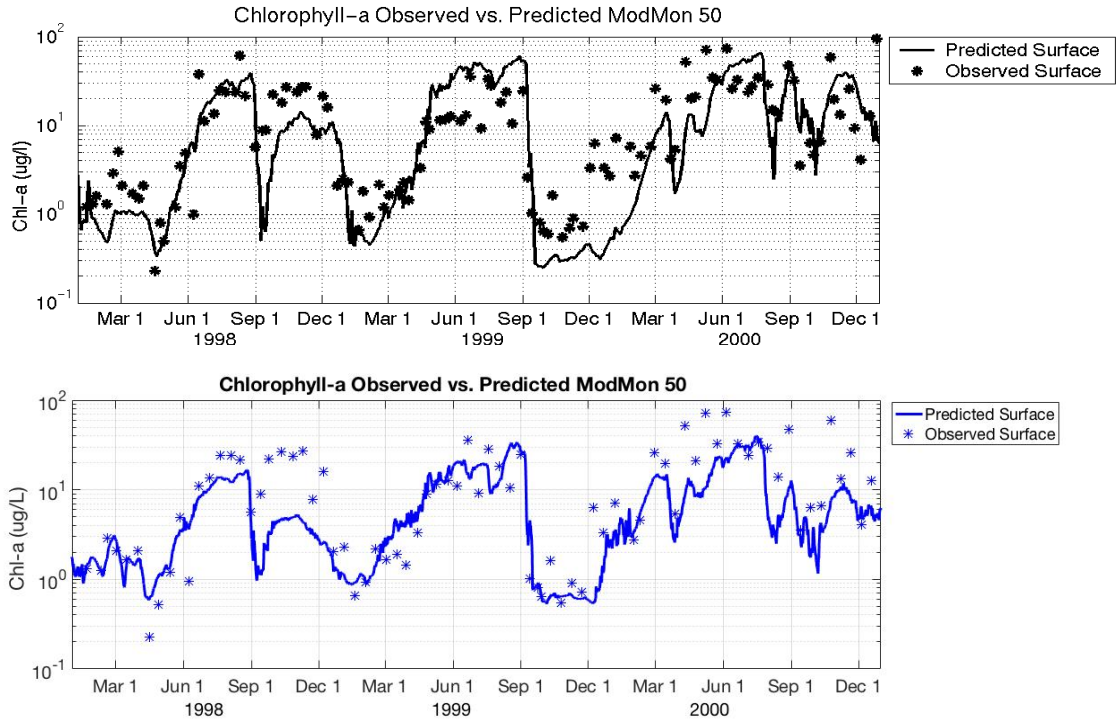

Figure 6. *Neuse Estuary Eutrophication Model predictions (solid line) and observed data (symbols) for surface conditions at ModMon station 50 for the original model (upper panel) and the updated model (lower panel).*

A second model time period (2004-2009) was also used to compare the model's calibration performance to that of the original model and the new model simulating the original model time period. For the new model, both time periods (1997-2000 and 2004-2009) had similar calibration statistics for total dissolved solids (TDS), nitrate + nitrite (NOX), and dissolved oxygen (Figure 7), while there were some observed differences between the old and new model for ammonia, orthophosphate, and chlorophyll-a (Figure 7). It should be noted that these

comparisons were made as an initial test of the new model's capabilities. A more thorough calibration and testing phase of the project is now underway with the new model.

What we have found so far using the newly expanded model data set is the continuation of the trend in nutrient loading towards a higher fraction in the organic nitrogen form. This trend was observed by previous researchers using a long-term analysis of flow normalized nutrient concentrations in Neuse Estuary locations taken from ModMon data (Lebo, Paerl et al. 2012). We have performed a similar analysis using model input data to calculate trends in flow-normalized nitrogen loading to the estuary. Using ModMon water quality data and USGS flow information, we have estimated flow-normalized nitrogen loading for total inorganic nitrogen, total organic nitrogen, and algal organic nitrogen over a time period from June 1997 to June 2014. Over that time period the inorganic fraction of the total decreased from seventy to less than fifty percent while the organic fraction increased from twenty-five to more than fifty percent (Figure 8).

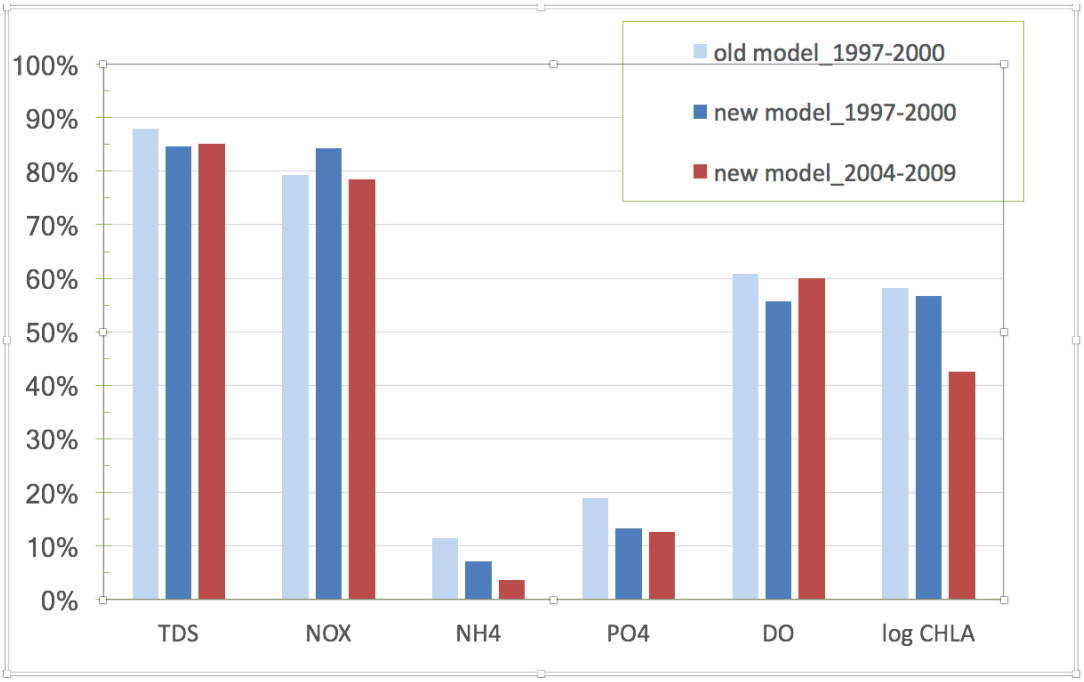

Figure 7. *NEEM Model predicted correlations coefficients to observed for total dissolved solids (TDS), nitrate + nitrite (NOX), ammonia (NH4), orthophosphate (PO4), dissolved oxygen (DO), and log base ten of chlorophyll-a concentrations (log CHLA) for the original NEEM and two simulation time periods of the updated NEEM.*

Over the 1997-2014 time period, organic loading to the estuary has more than doubled from approximately four million kg in 1997 to more than eight million kg in 2014 (Figure 9). Over this same time period the contribution of the nitrogen load from the algal organic component has remained relatively small and constant, while the inorganic load has actually decreased by approximately twenty percent from above eleven million kg in 1997 to an average of approximately nine million kg per year in the 2007-2012 time period (Figure 9). It should be noted, however, that the most recent year for which data are available (2014) shows an increase

in inorganic loading above the nine million kg annual value seen over the previous six years (Figure 9).

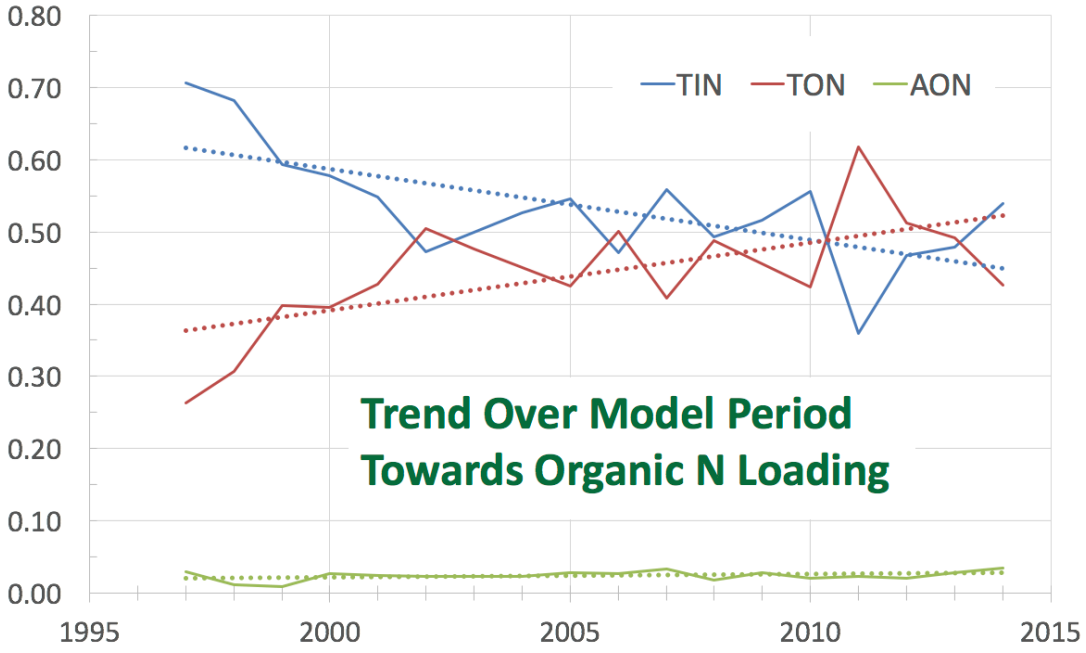

Figure 8. *Fractions of flow normalized nitrogen loading to the Neuse River Estuary in inorganic (TIN), organic (TON), and algal organic (AON) forms of nitrogen.*

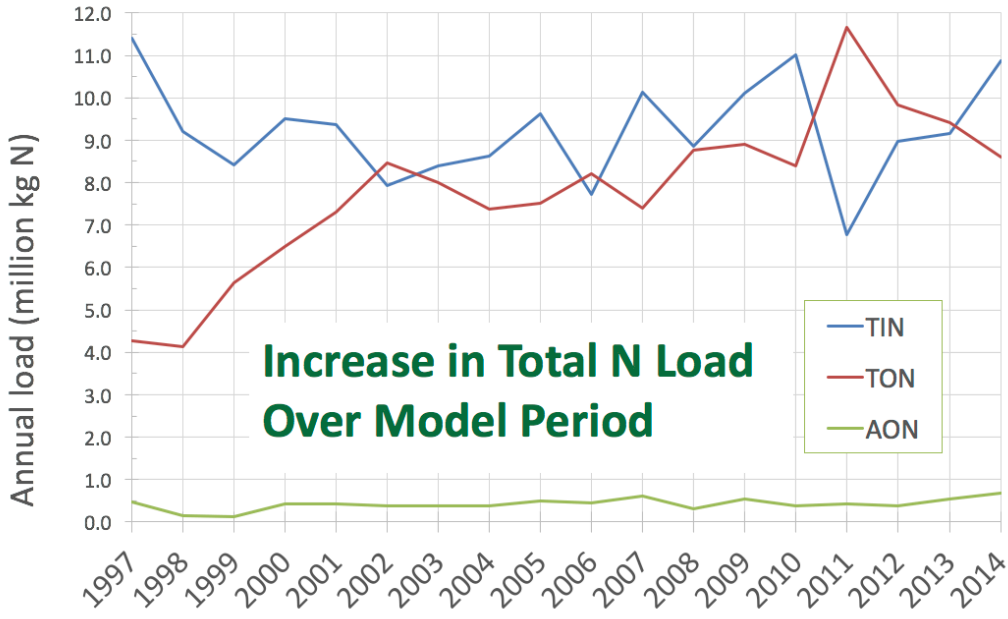

Figure 9. *Flow normalized nitrogen loading to the Neuse River Estuary in millions of kg per year for inorganic (TIN), organic (TON), and algal organic (AON) forms of nitrogen.*

3. Ongoing Work

We are currently working to finalize the calibration work on the model. The newly expanded model time period with many extreme weather events and algal blooms provides a wonderful opportunity to test the capability of the model to simulate the water quality dynamics in the system. The long time period also maximizes the differences in nitrogen loading that have been observed both in the magnitude of the load and the fraction that is in inorganic vs. organic forms. We expect to complete the testing phase of the model in May. We will begin immediately thereafter to begin the scenario testing phase of the model project. Overall the project is on schedule to be completed by August 31, 2017.

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How, where, when, and why: Defining Eutrophication Related Trends in Water Quality for the Middle and Lower Cape

Basic Information

Title:	How, where, when, and why: Defining Eutrophication Related Trends in Water Quality for the Middle and Lower Cape
Project Number:	2016NC202B
Start Date:	3/1/2016
End Date:	2/28/2017
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Congressional District:	NC-03
Research Category:	Water Quality
Focus Category:	Water Quality, Hydrology, Nutrients
Descriptors:	None
Principal Investigators:	Nathan S Hall, Hans W Paerl

Publications

There are no publications.

A Progress Report to the

Water Resources Research Institute

of

The University of North Carolina

On NC WRRI Project # 16-04-W, Entitled

"How, where, when, and why: Defining Eutrophication Related Trends in Water Quality for the Middle and Lower Cape Fear River Basin"

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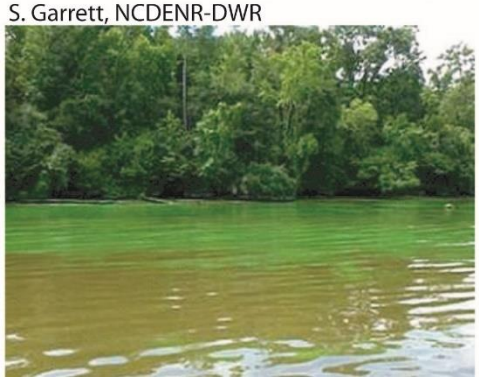
Date Submitted: 25 May 2017

1. **Background:** Over the last ten years, water quality conditions in the Cape Fear River (CFR) appear to be worsening with perceived intensification of eutrophication related symptoms, including algal blooms and occurrence of hypoxia and low pH (NCDENR 2009). Several segments of the CFR and its tributaries have been designated as "impaired" due to violations of State standards for chlorophyll *a* (Chl), dissolved oxygen (DO), and/or pH (NCDENR 2009). Of particular concern are blooms of toxigenic cyanobacteria, *Microcystis* spp, that have become a recurrent summertime problem since 2009. The blooms produce toxins (Isaacs et al. 2014), and taste/odor compounds that threatens the value of the CFR as a supply of potable water for > 0.5 million residents in some of the NC's most rapidly growing counties (NC-OSBM 2009). Additionally, the visually unappealing surface scums produced by the blooms curtail recreational use, and decaying bloom organic matter may exacerbate low DO and pH conditions (Mallin et al. 2006). Low DO and pH threaten the value of the river as habitat for aquatic life, including thirty State or federally listed endangered or threatened species (Cape Fear Action Plan 2009; NCDENR 2009). Low DO is thought to be partly responsible for declines in anadromous fish populations, including recreationally and commercially valuable species such as striped bass and herring (CFRP 2013).

Figure 1. Microcystis bloom on the Cape Fear R. (station B8349000, Aug 2012). Photo by S. Garrett, NCDENR-DWR



Observed water quality impairments (high Chl, low DO and pH) in the CFR have been ascribed to eutrophication caused by excessive anthropogenic nutrient inputs (N and P) (Mallin et al. 2006; Kennedy and Whalen 2008). Thus, there is a general perception that water quality is deteriorating in the CFR due to eutrophication. However, basin-wide, comprehensive trend analyses of eutrophication related water quality parameters such as phytoplankton biomass (as Chl *a*), DO, pH, water transparency, and nutrients (N and P) that may fuel eutrophication have not been conducted (see Related Research). Therefore, it cannot be said with certainty how conditions have changed, much less assess the magnitude or explore possible causes of change. This project is providing the first spatially and temporally comprehensive, and scientifically robust analysis of temporal trends and spatial patterns of in-stream concentrations and loads of eutrophication related water quality parameters for the CFR.

2. Research Question and Objectives: Trends in concentrations and fluxes of key eutrophication related parameters, including N and P nutrient forms (ammonium, nitrate, organic N, TN, & TP), phytoplankton biomass as Chl, total suspended sediments (TSS), water clarity as Secchi disk depth (SD), pH, and DO will be assessed at nineteen carefully selected stations within the middle and lower CFR basin (Table 1, Figure 2). Questions and objectives are designed to produce an ecosystem-level description of how human and climatic impacts have affected water quality over time as water is transported downstream through the watershed.

Table 1. Description of water quality records selected for trend analyses.

Water Body	Location	Station	Period	Parameters	USGS Gage #
CFR	Corinth	B616[2]	1995-	Nut[1] TSS Cond[5] DO pH Chl	02098206+02102[2]
	Lillington	B637	1972-	Nut TSS Cond DO pH	021025
	Fayetteville	B760	1992-	Nut TSS Cond DO pH	021055-021042
	Above Lock & Dam 3	B829	1998-	Nut TSS Cond DO pH Chl SD[3]	021055
	Below Lock & Dam 3	B8302	1998-	Nut TSS Cond DO pH SD	021055
	Tarheel	B8305	1992-	Nut TSS Cond DO pH Chl SD	021055
	Lock &Dam 2	B8339	1998-	Nut TSS Cond DO pH Chl	021055
	Above Lock & Dam 1	B8349	1998-	Nut TSS Cond DO pH Chl SD	02105769
	At Lock & Dam 1	B835	1973-	Nut TSS Cond DO pH	02105769
	Below Lock & Dam 1	B836	1996-	Nut TSS Cond DO pH Chl	02105769
	Neils Eddy	B845	1991-	Nut TSS Cond DO pH	02105769
CFR Estuary	C.M. 61 Wilmington	B9800	1972-	Nut TSS DO pH Chl SD	salinity[4]
Tributaries					
Haw R.	Moncure	B408	2000-	Nut TSS Cond DO pH	02098206
Deep R.	Moncure	B604	2000-	Nut TSS Cond DO pH	02102
Buckhorn Cr.	Corinth	B6204	2005-	Nut TSS Cond DO pH SD	02102192
Little R.	Manchester	B728	1977-	Nut TSS Cond DO pH	02103
Rockfish Cr.	Raeford	B770	1998-	Nut TSS Cond DO pH SD	0210422
Black R.	Tomahawk	B8750	1974-	Nut TSS Cond DO pH	021065
NE CFR	Sarecta	B91915	1996-	Nut TSS Cond DO pH	02108

[1]Nut=nutrients including TN, TKN, NO_3^-+NO_2^-, NH_4^+, and TP. [2] Trailing 0s omitted. [3]Secchi depth.[4]See methods. [5]Conductivity

Question 1: How have water quality conditions changed over the past decades in the CFR? Are eutrophication related water quality parameters increasing or decreasing? What is the magnitude of observed change?

Objective 1: Conduct traditional, non-parametric trend analyses for key eutrophication related water quality constituents at representative sites within the middle and lower CFR basin. Trend analysis for each station and each water quality constituent will provide a statistically-robust and scientifically defensible determination of the direction and magnitude of change, and associated degrees of uncertainty. Analyses will formally be used to test the null hypothesis that no long-term change has occurred.

Figure 2. Map of stations and flow gages for water quality trend analyses.

Question 2: Where are changes in water quality most apparent within the basin? Are there specific stations or regions within the main-stem CFR and tributaries where water quality is improving or deteriorating more rapidly than others. Land-use

development, changes in point sources, and water quality management actions vary within the basin (NCDENR 2005) and assessing spatial differences in trends along the main stem and within the tributaries will be important for understanding how these changes in the watershed have influenced water quality.

Objective 2: Compare the spatial distribution of trends in the direction and magnitude of changes. Spatial analysis and comparisons of trends will help identify "hot spots" that contribute to system-wide changes in water quality.

Question 3: When within the data records are changes most apparent? Changes in concentrations and fluxes rarely exhibit smooth trajectories. Rather, they typically respond to human or climatic influences that result in break-points where annual averages, seasonal patterns, or relationships with flow can change abruptly.

Objective 3: Use a recently developed weighted-regression trend analysis technique to produce time-varying models of concentrations and flux. Traditional trend analysis models assume that the long-term trend, seasonal pattern, and relationship with flow are constant throughout the data record (Hirsch et al. 2010). The newer weighted-regression model technique is capable of capturing variations in both long-term and seasonal patterns of time and changes in relationships between concentrations and flow. By capturing these three effects, the model can determine during which years, which seasons, and what flow regimes (i.e. low, base-flow conditions or high flow) shifts in water quality occurred.

Question 4: Why have conditions changed? From a management perspective understanding why conditions have changed is critical for making informed decisions about how to protect water resources. If conditions are improving, then understanding why will help replicate success. If conditions are worsening, then understanding why will help guide development of effective strategies for restoring or stabilizing water quality.

Objective 4: Compare information on how, where, and when (year, season, and flow regime) changes have occurred with information on known changes in point sources, land-use change, agricultural practices, and hydrology in the basin. Seasonality and the relationship with flow are largely determined by the sources of the constituent (i.e. point source, overland runoff, or groundwater) and instream transformational processes (i.e. sedimentation, denitrification, biological uptake/ production) (Behrendt 1999; Hirsh et al. 2010; Moyer et al. 2012; Beck and Hagy 2015). Detecting shifts in these behaviors (Objective 3) will provide important clues to how sources or transformational processes have changed, and by doing so, help identify causes of long-term trends in water quality (Alameddine et al. 2011; Sprague et al. 2011; Hirsch et al. 2010). Relating observed trends and shifts in constituent behavior to human and/or hydrologic changes in the basin can provide valuable information on changes in sources or effects of restoration efforts that are responsible for trends (Sprague et al. 2011; Beck and Hagy 2015).

3. Project Results to Date:

Water quality records and accompanying river flow data have been downloaded and formatted for trend analyses using both Seasonal Kendal Tests and Weighted Regressions on Time, Discharge, and Season. Seasonal Kendal Tests on flow corrected concentrations have been conducted for all parameters at all stations for approximately the last fifteen to twenty five years of the data records. This period corresponds to the time frame of the beginning of sampling for many of the stations sampled by the Middle Cape Fear River Basin Association and the Lower

Cape Fear River Project. For sites sampled by the North Carolina Division of Water Quality (DWQ), a five year hiatus of nutrient sampling occurred from 1986 to 1991. For comparing spatial patterns of trends at stations sampled by all three organizations it was deemed more appropriate to initially analyze only the DWQ data collected after 1991. Table 2 shows the time frame of data records analyzed so far for each of the nineteen stations.

Table 2. Time frame of water quality records analyzed to date by Seasonal Kendal tests.

Water Body	Location	Station	Period
CFR	Corinth	B616	1992-2015
	Lillington	B637	1992-2016
	Fayetteville	B760	1992-2015
	Above Lock & Dam 3	B829	1998-2015
	Below Lock & Dam 3	B8302	1998-2015
	Tarheel	B8305	1992-2014
	Lock &Dam 2	B8339	1998-2015
	Above Lock & Dam 1	B8349	1998-2015
	At Lock & Dam 1	B835	1992-2015
	Below Lock & Dam 1	B836	1991-2014
	Neils Eddy	B845	1991-2012
CFR Estuary	C.M. 61 Wilmington	B9800	1991-2016
Tributaries			
Haw R.	Moncure	B408	2000-2015
Deep R.	Moncure	B604	2000-2015
Buckhorn Cr.	Corinth	B6204	2005-2014
Little R.	Manchester	B728	2003-2015
Rockfish Cr.	Raeford	B770	1992-2015
Black R.	Tomahawk	B8750	1992-2015
NE CFR	Sarecta	B91915	1998-2015

Results to date have largely focused on addressing questions 1 and 2 and fulfilling objectives 1 and 2. Seasonal Kendall-Mann tests have been performed for all the water quality records shown in Table 1 for the time frames at each station shown in Table 2. Seasonal Kendall-Mann tests were performed on flow corrected concentrations to detect and quantify whether significant, monotonic long-term trends exist within each data series. The Kendal-Mann test (Hirsch et al. 1982) on flow corrected values is the standard test used by NCDENR-DWR (2005) and removes influences of flow dependency and seasonality to provide a statistically robust test of the null hypothesis that concentrations and loads exhibit no trend with time. Flow correction was achieved by calculating the residuals from a LOESS regression of concentration on flow according to (Aroner 2000) and NCDENR DWR trend analysis practices (Rajbhandari 2004). Accounting for variation in concentrations due to flow and seasonality reduces uncertainty in model coefficients for the long-term trend and thus decreases the probability of not detecting a

trend when a trend actually exists (Hirsch et al. 1982; Cohn et al. 1989). For each water quality parameter, the seasonal Kendall-Mann test was used to test the null hypothesis that the parameter exhibits no long-term trend through time. P-values were adjusted to account for autocorrelation during hypothesis testing (Hirsch and Slack 1984). When significant positive or negative long-term trends was determined, Sen slopes were calculated to quantify the average magnitude of change over each data record (Hirsch et al. 1982). This traditional non-parametric method of trend analysis provides a robust determination of the direction and average magnitude of water quality change within a data record, is insensitive to non-normality or small percentages of censored data. An example of the flow correction and Seasonal Kendall-Mann analyses on flow corrected values is shown for the total N record at station B728 on the Little River near Manchester, NC. In this case, a clear decreasing trend of TN is evident and contrasts strongly with the general pattern of increasing TN concentrations throughout the basin as shown below.

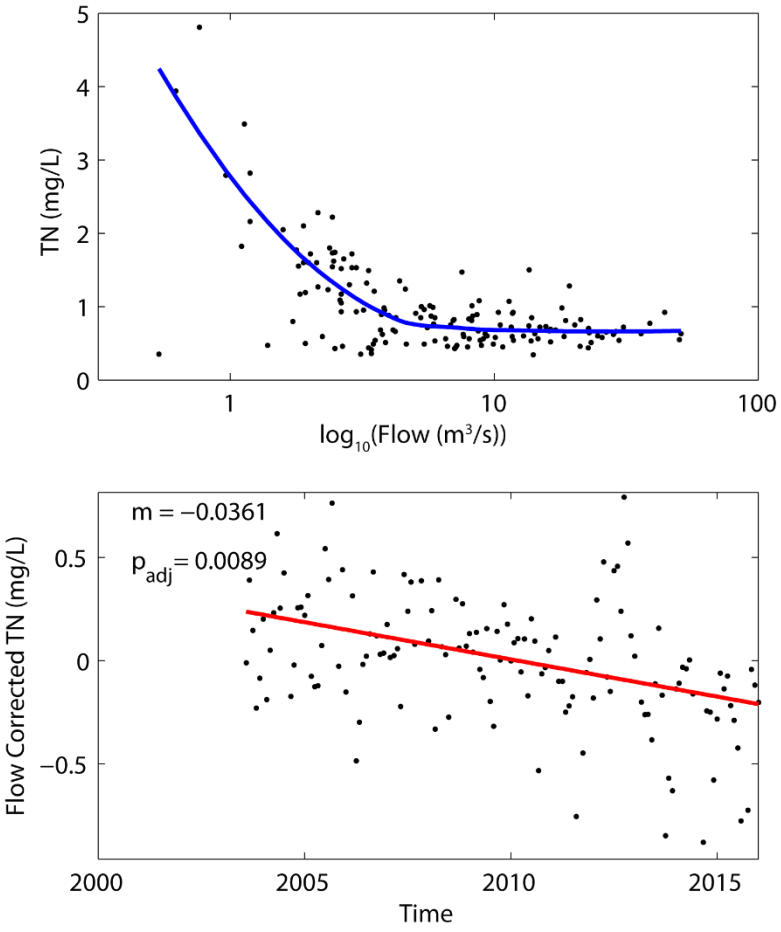


Figure 3. Example of flow correction using a LOESS regression and Seasonal Kendal test of flow corrected values for total N data collected at station B728 on the Little River near Manchester, NC. m values are Sen slopes in units mg/L/y. P values are adjusted for serial correlation.

To illustrate spatial patterns of change, magnitudes and directions (increasing or decreasing) of statistically significant Sen Slopes were mapped for each parameter at each station. Spatial coherence in the directions and magnitudes of observed trends increases confidence in the validity of these trend assessments, the underlying data sources, and provides information to link observed changes to human activities within the basin.

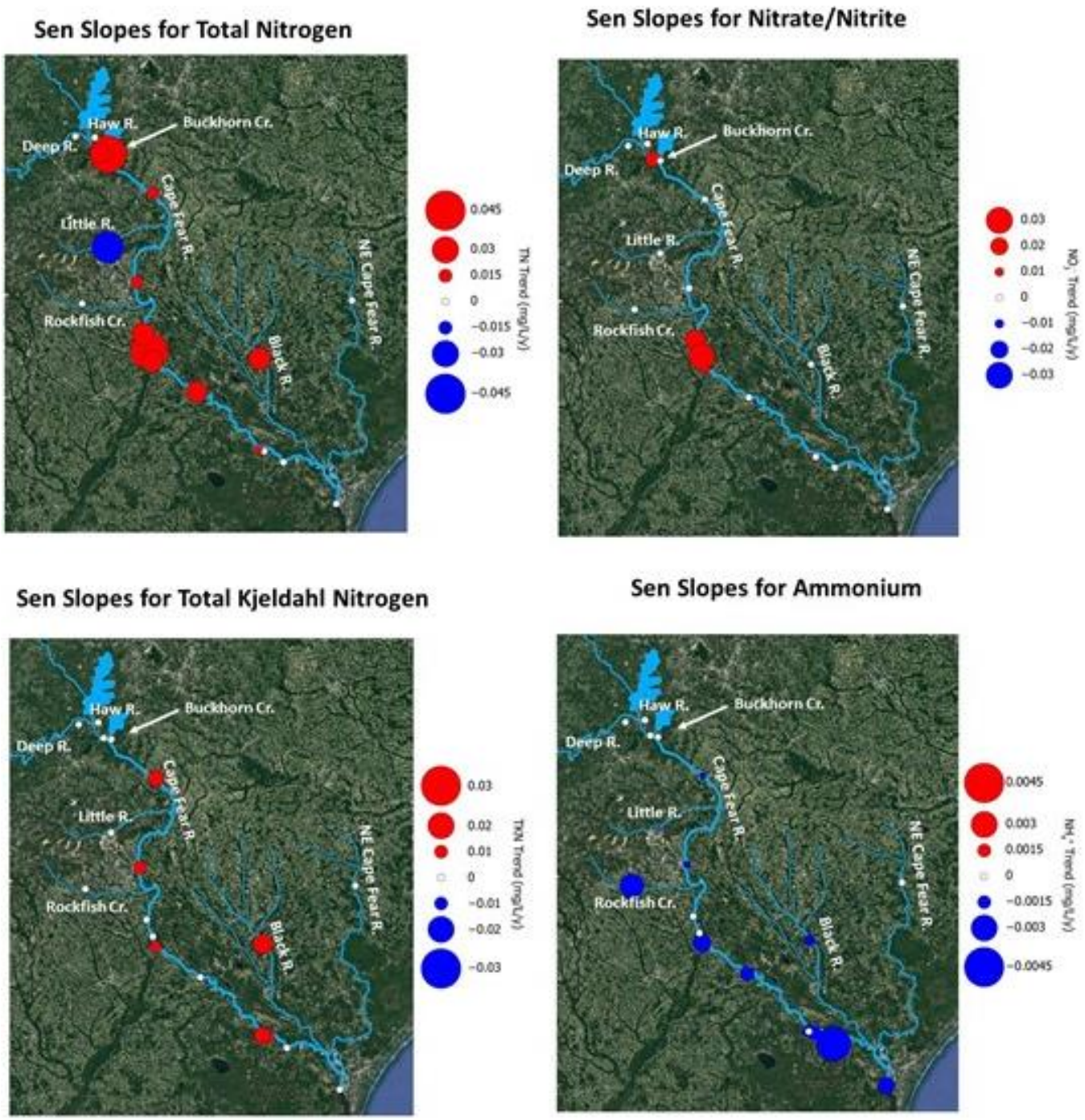


Figure 4. Summary map of the magnitude and direction of trends in nitrogen species in the middle and lower Cape Fear River basin. White circles indicate that no significantly significant trend was detected

Increasing trends in total nitrogen, nitrate, and total Kjeldahl nitrogen (primarily organic N) were detected and many stations within the basin. The largest increase was observed in Buckhorn Cr. but this small tributary (average flow only 0.25 m^3/s) is unlikely to have strong impacts on the condition of the main stem of the Cape Fear River. Strong increasing trends in both total N and nitrate in the Cape Fear River downstream of Fayetteville were also observed. This region is impacted by the highest loads of waste water treatment effluent within the study area (TetraTech 2015). Project year 2 efforts to analyze point source discharge data and determine flow relatedness of nitrogen species will help determine whether increases in wastewater loads are responsible for these trends. Where significant trends were detected, ammonium showed a consistent pattern of decreasing concentrations. This finding contrasts previous conclusions of increasing ammonium concentrations within the basin (Burkholder et al. 2006). Similar decreasing trends in total phosphorus were detected at many stations throughout the basin (Figure 5) with the largest declines observed in the region below Fayetteville. Year two analyses will seek to determine the possible role of improved P removal in wastewater treatment on these trends.

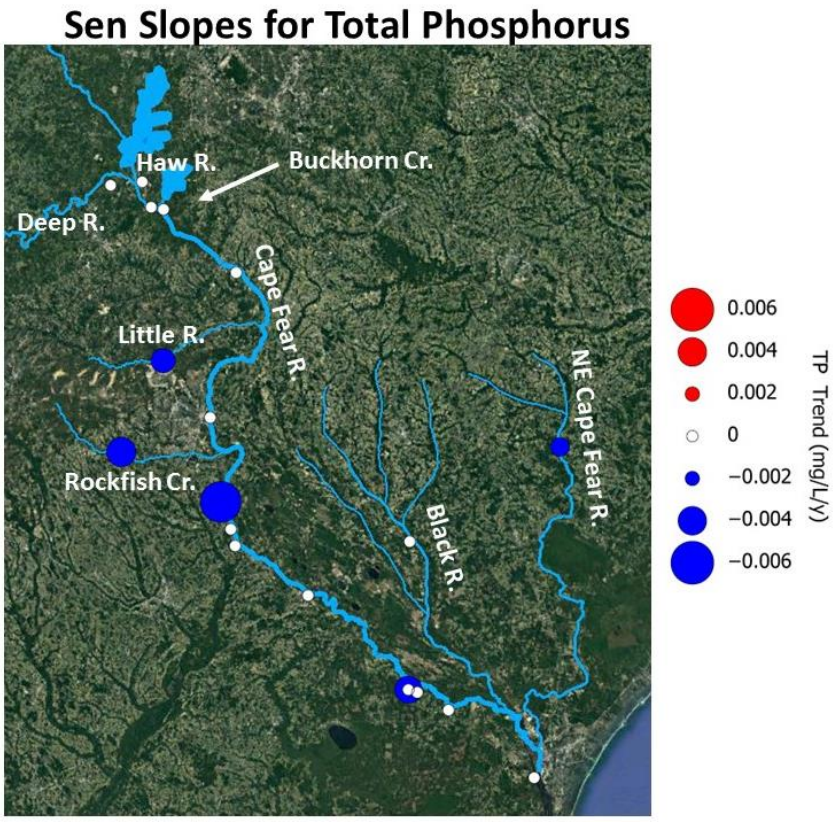


Figure 5. Summary map of the magnitude and direction of trends in Total Phosphorus in the middle and lower Cape Fear River basin. White circles indicate that no significantly significant trend was detected

Despite concerns over low oxygen conditions throughout the basin (Bowen and Rajbhandari 2012), increasing trends in dissolved oxygen were detected at many stations and no declining trends in dissolved oxygen were observed (Figure 6). pH exhibited significant decreases in the upper part of the basin but increased in the North East Cape Fear River (Figure 6). Conductivity increased significantly in the Black River, North East Cape Fear River and Buckhorn Cr (Figure 6). These trends are likely due to human activities in the watershed which will be explored further in project year 2. The large increase in conductivity observed within the Cape Fear River estuary is too large to be driven by changes in the watershed and is most likely driven by increases in salinity due to channel deepening and greater tidal exchange in the North Carolina Port at Wilmington. Increasing trends in total suspended solids were observed in the lower reaches of the Cape Fear River and in the coastal plain tributary rivers, the Black River and North East Cape Fear River (Figure 6). Reasons for these trends in total suspended solids

will be further investigated during project year 2. Seasonal Kendal tests were conducted for Secchi depth and chlorophyll *a* for the stations listed in Table 1 but no significant trends were detected.

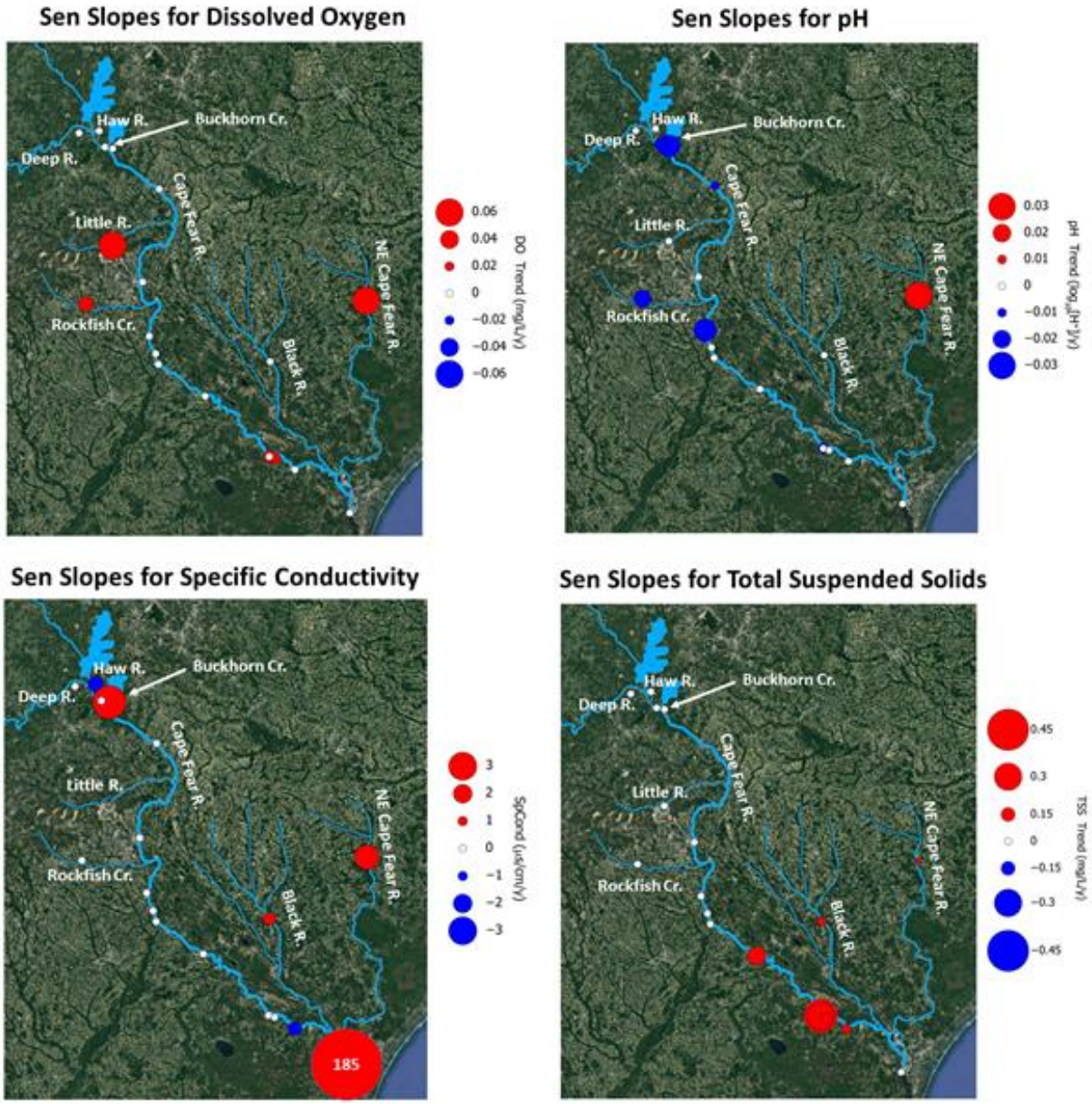


Figure 6. Summary maps of the magnitude and direction of trends in dissolved oxygen, pH, specific conductivity, and total suspended solids in the middle and lower Cape Fear River basin. White circles indicate that no significantly significant trend was detected. Slope for the trend in conductivity at estuarine station B980 is written on the symbol.

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Appendix I. List of Acronyms

Chl	chlorophyll *a*
L	liter
m	meter
m^{-3} s^{-1}	meters cubed per second
μg L^{-1}	micrograms per liter
MCFRBA	Middle Cape Fear River Basin Association
mg L^{-1}	milligrams per liter
mL	milliliter
N	nitrogen
NCDEQ	North Carolina Department of Environmental Quality
NH_4^+	ammonium
NO_2^-	nitrite
NO_3^-	nitrate
TP	total phosphorus
PO_4^{-3}	orthophosphate
TSS	total suspended solids
UNC-IMS	University of North Carolina at Chapel Hill's Institute of Marine Sciences
USGS	U.S. Geological Survey
WRTDS	Weighted Regressions on Time Discharge and Season
y	year

Appendix II.

Information Transfer: Project results and findings are being widely dessimated to managers, stakeholders and fellow scientists with interests in the water quality of the Cape Fear River Basin. On 17 March 2016 PI Hall presented a poster at the NC WRRI Annual Conference 2016, Raleigh entitled ""Leveraging cutting-edge techniques to determine drivers of water quality in the Cape Fear River." This poster provided an overview of the problem, project goals and objectives, and methods and some preliminary results from trend analyses. PI Hall gave an oral presentation on 20 April 2016 to North Carolina's Nutrient Criteria Development Plan's Scientific Advisory Council entitled "Determining Water Quality Change and Drivers on the Middle Cape Fear River: An Introduction to Two New Projects". The presentation covered the goals and objectives of this project and a NC Seagrant project on Microcystis bloom dynamics and presented preliminary findings of trends in nutrient concentrations along the Middle Cape Fear River. PI Hall gave a similar talk was given the Middle Cape Fear River Basin Association's quarterly meeting on 4 May 2016. The Basin Association is an organization comprised primarily by the dominant point sources within the basin. On 16 March 2017, PI Hall gave an oral presentation at NC WRRI's annual conference in Raleigh entitled "Unraveling dual influences of increasing nutrients and changing flow regimes on bloom potentials along the middle Cape Fear River ". The talk focused on the role of changing water quality and water quantity conditions in relation to drivers of cyanobacteria blooms on the Cape Fear River.

Tracing Groundwater Contamination near Coal ash Ponds in North Carolina

Basic Information

Title:	Tracing Groundwater Contamination near Coal ash Ponds in North Carolina
Project Number:	2016NC203B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NC-004
Research Category:	Water Quality
Focus Category:	Water Quality, Hydrogeochemistry, Toxic Substances
Descriptors:	None
Principal Investigators:	Avner Vengosh

Publication

1. Vengosh, A., Coyte, R., Karr, J., Harkness, J.S., Kondash, A.J.*, Laura S. Ruhl, L.A., Rose B. Merola, R.B., Dywer, G.S. (2016) The Origin of Hexavalent Chromium in Drinking Water Wells from the Piedmont Aquifers of North Carolina. Environmental Science & Technology Letters, 3 (12), 409–414.

Environmental Science & Technology Letters

Letter

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Origin of Hexavalent Chromium in Drinking Water Wells from the Piedmont Aquifers of North Carolina

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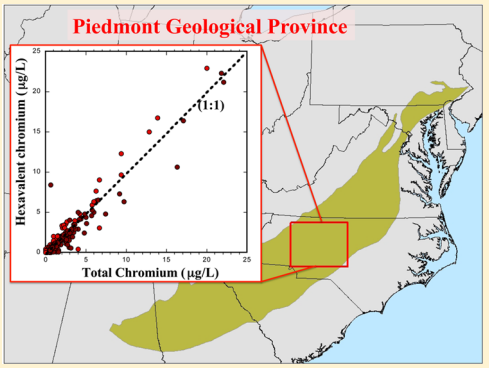
Supporting Information

ABSTRACT: Hexavalent chromium [Cr(VI)] is a known pulmonary carcinogen. Recent detection of Cr(VI) in drinking water wells in North Carolina has raised public concern about contamination of drinking water wells by nearby coal ash ponds. Here we report, for the first time, the prevalence of Cr and Cr(VI) in drinking water wells from the Piedmont region of central North Carolina, combined with a geochemical analysis to determine the source of the elevated Cr(VI) levels. We show that Cr(VI) is the predominant species of dissolved Cr in groundwater and elevated levels of Cr and Cr(VI) are found in wells located both near and far (>30 km) from coal ash ponds. The geochemical characteristics, including the overall chemistry, boron to chromium ratios, and strontium isotope ($^{87}Sr/^{86}Sr$) variations in groundwater with elevated Cr(IV) levels, are different from those of coal ash leachates. Alternatively, the groundwater chemistry and Sr isotope variations are consistent with water−rock interactions as the major source for Cr(VI) in groundwater. Our results indicate that Cr(VI) is most likely naturally occurring and ubiquitous in groundwater from the Piedmont region in the eastern United States, which could pose health risks to residents in the region who consume well water as a major drinking water source.



INTRODUCTION

Since the early findings of hexavalent chromium [Cr(VI)] in drinking water in the Hinkley community of San Bernardino County, California, presumably from Cr(VI) additives at water-cooling towers from gas compressor facilities, there has been a persistent controversy about the sources of Cr(VI) in groundwater and its human health impacts.[1] Most Cr in aquatic systems occurs as either the trivalent chromium [Cr(III)] cation Cr^{3+} or Cr(VI) oxyanions, such as the monovalent $HCrO_4^-$ and divalent CrO_4^{2-} species.[2−4] All Cr(VI) compounds are strong oxidizing agents and are recognized by the World Health Organization (WHO) as "carcinogenic to humans (Group 1)",[15] and Cr(VI) is recognized as a pulmonary carcinogen.[5−14] However, the U.S. Environmental Protection Agency (EPA) does not regulate individual Cr species, and the drinking water standard includes only total Cr [Cr_T, maximum contaminant level (MCL) of 100 µg/L];[4] the most updated 2003 WHO guidelines for drinking water include only total Cr with an upper limit of 50 µg/L.[15] The absence of Cr(VI) from the drinking water regulations was explained by analytical limitation and the assumption that the speciation of Cr favors the predominance of the less toxic Cr(III) under typical environmental conditions.[4,15] To date, only the state of California has issued a specific MCL of 10 µg/L and a public health goal (PHG) of 0.02 µg/L for Cr(VI) in drinking water.[16]

It is commonly assumed that the occurrence of Cr(VI) in drinking water wells is directly associated with human activities, and any detection of Cr(VI) infers anthropogenic contamination.[4,15] However, recent reports have established that naturally occurring Cr(VI) is prevalent in groundwater from specific aquifer systems composed of ultramafic rocks, known to be enriched with Cr relative to other rock types.[17] Elevated Cr(VI) levels were reported in groundwater associated with ultramafic aquifers in California,[18−21] Arizona,[22] Mexico,[23] Argentina,[24] Brazil,[25] Italy,[26] and Greece.[27] Experimental work demonstrated that the presence of manganese oxide minerals within ultramafic- and serpentinite-derived soils and/or sediments can trigger the oxidation of Cr, leading to the presence of naturally occurring Cr(VI) in aquifers.[28]

Recent detection of Cr(VI) in drinking water wells near coal ash ponds in North Carolina[29] has been attributed to leaking from nearby coal ash ponds because an elevated Cr levels have been reported in coals and coal ash residuals (CCRs).[30−33] This

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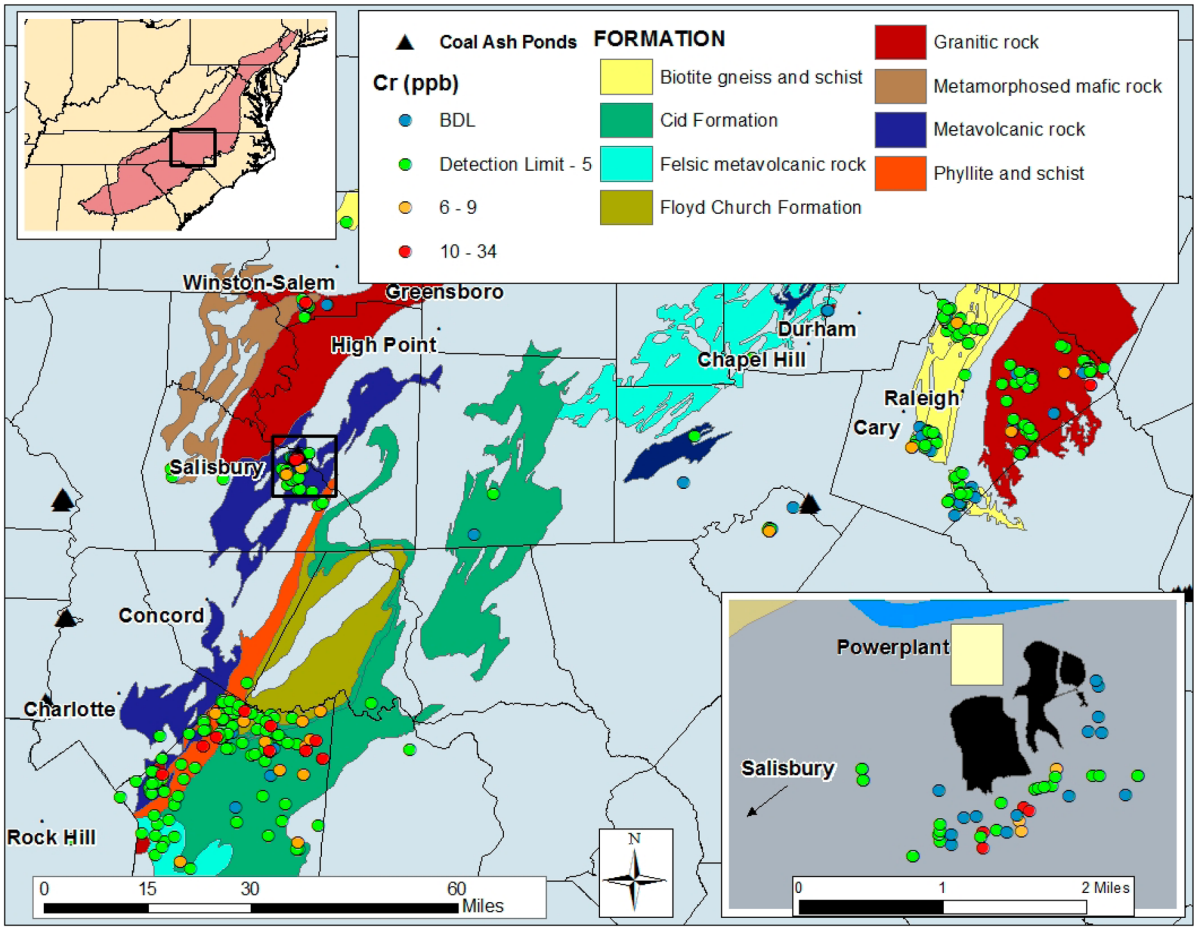

Figure 1. Distribution of total Cr concentrations (ranked by color, in micrograms per liter) in drinking water wells, coal ash ponds, and selective geological formations in the Piedmont region of North Carolina. The small inset map at the top left shows the distribution of the Piedmont geology in the southeastern United States. The bottom inset map shows the distribution of Cr near coal ash ponds close to Salisbury, NC. The felsic metavolcanic rock and granitic rock categories are primarily felsic formations. The Cid, Floyd Church, metamorphosed mafic rock, and metavolcanic formations are of mixed character with varying levels of mafic components. The biotite gneiss and schist and phyllite and schist categories are characterized as general metamorphic bodies. The Cr concentrations in groundwater from the different formations are reported in Table S2. Geological data and location of coal ash ponds were retrieved from U.S. Geological Survey database[44] and Southern Alliance for Clean Energy.[45]

study aims to determine whether coal ash ponds are causing the Cr(VI) contamination in local aquifers or if Cr(VI) is naturally occurring and ubiquitously distributed in groundwater across the Piedmont region. The study is based on systematic measurements of Cr_T and Cr(VI) in groundwater from different aquifers and varying distances from coal ash ponds in the Piedmont region of North Carolina, combined with geochemical and strontium isotope tracers known to be indicative of coal ash contamination and water−rock interactions.[34−36] Previous studies have observed elevated Sr (>150 μg/L) and B (>100 μg/L) levels and distinct Sr isotope ratios ($^{87}Sr/^{86}Sr$ = 0.7095−0.7120) in effluent discharged from coal ash ponds and in contaminated surface and groundwater.[34−36] The Sr and B tracers are particularly useful for delineating the release of coal ash pond water because they are sensitive to very small contributions of contaminated water to the environment.[35,36] We hypothesize that Cr(VI) contamination from coal ash ponds will be associated with modification of the chemical and isotope compositions of the groundwater toward a coal ash geochemical signature.[34−36]

MATERIALS AND METHODS

Water samples in the study were collected from domestic groundwater wells in central North Carolina and were analyzed for major and trace elements (n = 376). A subset of these groundwater samples were analyzed for Cr(VI) (n = 77) and stable isotopes of strontium ($^{87}Sr/^{86}Sr$; n = 45). Water samples were collected before any treatment systems following standard methods.[37] Anions were measured by ion chromatography (IC) on a Dionex IC DX-2100 instrument; major cations were measured by direct current plasma optical emission spectrometry (DCP-OES) and trace elements by a VG PlasmaQuad-3 inductively coupled plasma mass spectrometer (ICP-MS). The DCP and ICP-MS instruments were calibrated to the National Institute of Standards and Technology 1643e standard. The detection limit of ICP-MS for each element was determined by dividing 3 times the standard deviation of repeated blank measurements by the slope of the external standard. Cr(VI) was measured as chromate according to a modified version of U.S. EPA Method 218.6.[38] This method is based on anion exchange chromatography on a Thermo Scientific Dionex IonPac AS7 column (4 mm × 250 mm) with a method detection limit (MDL) for chromate of 0.004 μg/L and a reporting limit of 0.012 μg/L (see the text of the Supporting

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Information). Strontium isotopes were analyzed by thermal ionization mass spectrometry (TIMS) on a ThermoFisher Triton instrument at Duke University. The external reproducibility of $^{87}Sr/^{86}Sr$ ratios was comparable to standard NIST987 (0.710265 ± 0.000006).

Geospatial analysis of data was conducted using ArcMap version 10.3.1. The background on the hydrogeology and geological map is provided in the text of the Supporting Information. Statistical analyses were performed using R (version 3.2.0). All correlations were Spearman nonparametric correlations, and the reported r is the Spearman rank coefficient, rho. The nonparametric Wilcoxon rank sum test was used to determine if concentration mean ranks differ between different populations.

RESULTS AND DISCUSSION

Geochemical Characteristics of Piedmont Groundwater. Total Cr (Cr_T) concentrations ranged from below the reporting limit (0.0016 μg/L) to 33.8 μg/L (Figure 1). In the subset of samples (n = 77) analyzed for Cr(VI), Cr(VI) concentrations varied from below the reporting limit (0.012 μg/L) to 22.9 μg/L and were highly correlated to Cr_T [slope of ~1; r^2 = 0.93; p < 0.001 (Figure 2)]. Our data are consistent with data reported by the North Carolina Department of Environmental Quality[25] for residents near coal ash impoundments (n = 129) that show the same range of Cr(VI) concentrations and a high correlation between Cr(VI) and Cr_T [slope of ~0.9; r^2 = 0.90; p < 0.001 (Figure 2)]. The average $Cr(VI)/Cr_T$ ratio of ~1 indicates that Cr(VI) is the predominant species of dissolved Cr in groundwater and accounts for nearly all of the dissolved Cr. While the NC-DEQ data are restricted to wells located near coal ash ponds, our data collection included wells located far (up to 75 km) from coal ash impoundments (Figure 1).

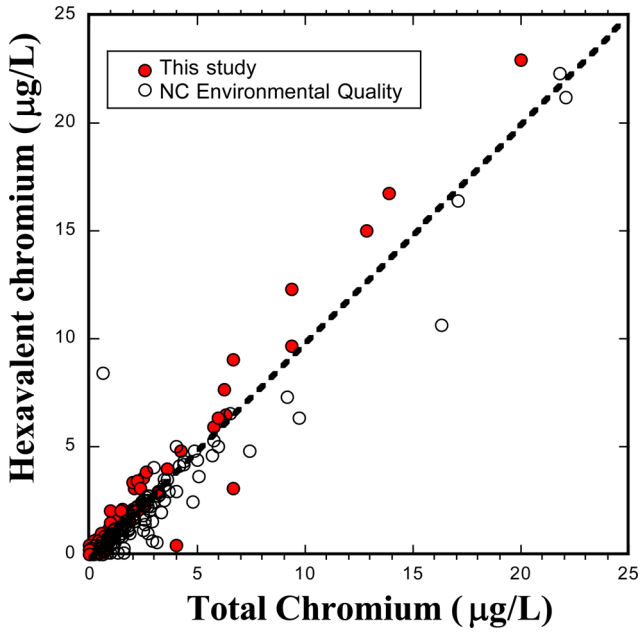

Figure 2. Hexavalent chromium concentration vs total Cr concentration in groundwater analyzed in this study (red circles) and reported by the North Carolina Department of Environmental Quality[25] (○). Note the high correlation of Cr(VI) to Cr_T in both data sets with an r^2 of 0.93 (p < 0.001; n = 77) reported in this study and an r^2 of 0.90 (p < 0.001; n = 129) in NC-DEQ data. The ~1:1 ratio in most of the samples indicates that Cr(VI) is the predominant species of dissolved Cr in the Piedmont groundwater.

Strontium concentrations in the groundwater ranged from the detection limit (0.25 μg/L) to 3426 μg/L, with low Sr/Ca ratios [<0.006 (Figure 3A)]. Groundwater from a Cr(VI)-rich

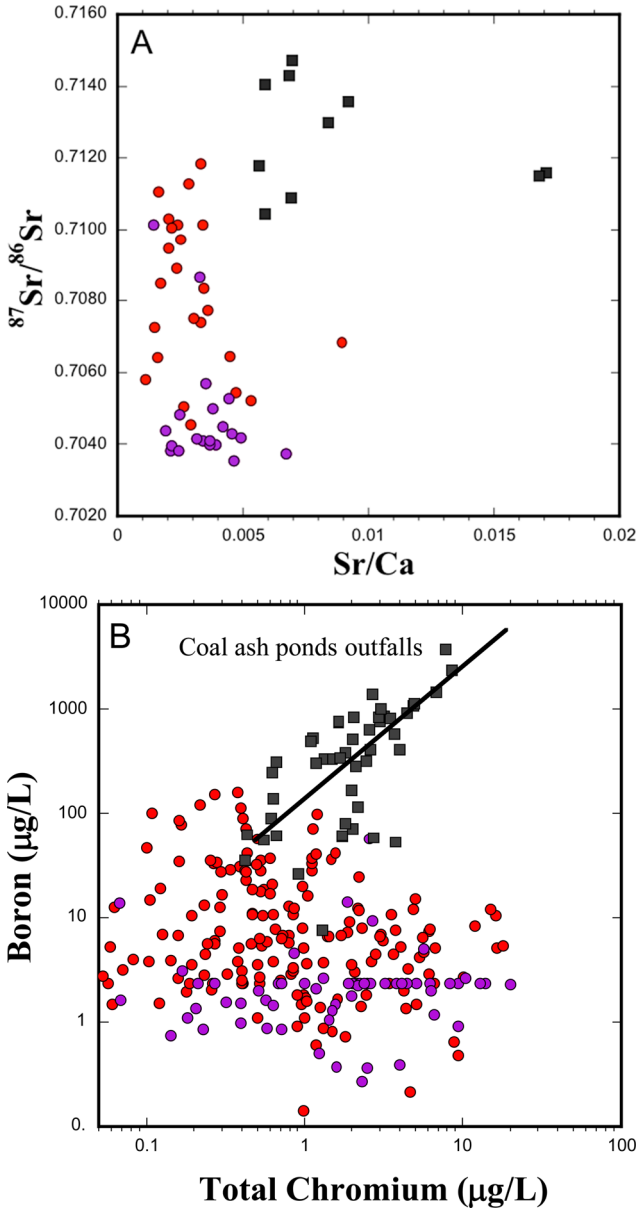

Figure 3. Variations of (A) $^{87}Sr/^{86}Sr$ vs Sr/Ca and (B) B vs total Cr (log scale) in groundwater from the Piedmont region (red and purple circles) as compared to that of effluent discharge from coal ash ponds' outfalls in North Carolina (black squares; data from ref 31). The data show systematically lower $^{87}Sr/^{86}Sr$, Sr/Ca, and B/Cr ratios in groundwater than in coal ash effluents. Groundwater from aquifers composed of metavolcanic rocks (purple circles) is characterized by distinctively lower $^{87}Sr/^{86}Sr$, Sr/Ca, and B contents relative to those of groundwater from other aquifers and coal ash effluents. The combined data indicate that the chemistry of the Piedmont groundwater is different from the composition of coal ash waters, particularly for groundwater from metavolcanic aquifers that are located near coal ash ponds (n = 16), thus ruling out the possibility of the contamination of drinking water wells by coal ash ponds.

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metavolcanic aquifer in Rowan County located near a coal ash pond (n = 16) (Figure 1) and aquifers containing mafic rocks from other counties in the Piedmont region (n = 7) had low $^{87}Sr/^{86}Sr$ ratios [0.7041 ± 0.0005 (Figure 3A)]. Groundwater from the other aquifers showed large variations in $^{87}Sr/^{86}Sr$ (a range of 0.7052−0.7119), with higher $^{87}Sr/^{86}Sr$ ratios in the felsic and phyllite and schist aquifers [0.7074−0.7119 (Figure S3)]. Boron concentrations from the Piedmont region were low (a range of 0.09−159.2 μg/L, median of 3.7 μg/L) with low B/Cr_T ratios [median of 8.6 (Figure 3B)]. In particular, groundwater from wells from the metavolcanic aquifer near coal ash ponds with high Cr(VI) concentrations in Rowan County (see the inset map in Figure 1) had systematically low B concentrations [median of 2.3 ± 17.3 μg/L (Figure S4)] and low B/Cr_T ratios (<200).

Tracing the Source of Hexavalent Chromium. Previous studies have shown that coal ash effluents and coal ash-contaminated groundwater have high concentrations of B and Sr with distinctive radiogenic Sr isotope ratios, which are different in some cases from those in natural waters.[34−36,39−41] Waters impacted by CCR effluents typically have high B and Sr concentrations (above background levels of 100 and 150 μg/L, respectively), high Sr/Ca ratios (>0.006), and high $^{87}Sr/^{86}Sr$ ratios (>0.70975).[34−36] In shallow groundwater monitoring wells around coal ash ponds in North Carolina, the B levels reached 5000 μg/L.[36] The low B concentrations and B/Cr_T, Sr/Ca (<0.006), and $^{87}Sr/^{86}Sr$ ratios of the Cr(VI)-rich groundwater in this study, including wells located near (<5 km) a coal ash pond, are inconsistent with the geochemistry expected for CCR-impacted water (Figure 3A). The low $^{87}Sr/^{86}Sr$ ratios observed in the groundwater in Rowan County are consistent with a nonradiogenic Sr isotope composition that is typical for the mafic rocks that are prevalent in this aquifer, indicating that Sr is derived from water−rock interactions and not from coal ash pond contamination. Higher $^{87}Sr/^{86}Sr$ ratios were observed in felsic aquifers and other nonmafic aquifers (Figure S3); however, these aquifers were not associated with high Cr(VI) and in many cases were located far from coal ash ponds.

Data from coal ash pond effluents in North Carolina[34] show that B is strongly correlated to Cr_T [r^2 = 0.68; p < 0.001 (Figure 3B)], with high B/Cr_T ratios of ∼297. In contrast, all groundwater from the Piedmont region had much lower B concentrations and B/Cr_T ratios. In particular, groundwater wells in Rowan County near the coal ash pond with a high Cr(VI) concentration had B levels and B/Cr_T ratios 2−3 orders of magnitude lower than those of coal ash pond effluents from North Carolina (Figure 3B).

In addition to the groundwater in Rowan County, samples were collected from wells in formations containing mafic rocks located in counties that did not have any coal ash ponds. Elevated Cr(VI) concentrations were detected in wells from mafic-rich aquifers in Forsyth County that are located more than 30 km from a coal ash pond. These samples had elevated Cr(VI) concentrations of up to 10 μg/L with Sr/Ca (<0.006), B/Cr_T (<20), and $^{87}Sr/^{86}Sr$ ratios similar to those of the mafic-containing aquifer near the coal ash ponds in Rowan County.

It is important to note that coal ash effluents that discharge from coal ash ponds in NC have high concentrations of sulfate, arsenic, selenium, molybdenum, and thallium relative to those of natural waters,[34] which are not present in the groundwater near coal ash ponds tested in this study. Furthermore, the range of Cr_T concentrations found in coal ash effluents [0.4−8.6 μg/L (Figure S5)] is lower than those measured in nearby groundwater. Overall, the geochemical and isotopic data clearly indicate that the drinking water wells tested in this study are not impacted by CCR effluents, and therefore, the coal ash ponds are not a likely source of the elevated Cr_T and Cr(VI) concentrations found in the Piedmont groundwater. These results are further supported by the presence of Cr(VI)-rich groundwater that has similar geochemistry in wells located more than 30 km from a coal ash pond (Figure 1 and Figure S2). Total Cr concentrations were not strongly correlated with distance (r = 0.09) but showed a significant (p < 0.05) increase with distance from coal ash ponds, and concentrations of up to 34 μg/L were found in wells more than 50 km from the nearest coal ash pond. These results indicate that high Cr concentrations can be found in wells located far from coal ash ponds, which is inconsistent with the expected trend if coal ash ponds were the source of Cr contamination in nearby groundwater. The geospatial analysis therefore supports the conclusions drawn from the geochemical data.

Distribution of Chromium in the Piedmont Aquifers. While our geochemical analysis rules out contamination from nearby coal ash ponds, we present evidence of a geogenic source of Cr and Cr(VI) to drinking water aquifers. First, we show that Cr [and Cr(VI)] can be found throughout the different aquifers of the Piedmont region (Figure 1). Second, the association of $^{87}Sr/^{86}Sr$ ratios with aquifer lithology (Figure S3) indicates that the local aquifer rocks are the source of dissolved Sr and apparently Ca in groundwater, given the high correlation between Sr and Ca (Figure S6). Third, the association of Ca and Cr in groundwater from some aquifers (Figure S7) suggests that Cr, like Ca, is derived from water−rock interactions rather than an external (i.e., anthropogenic) source. The distribution of Cr_T varies among the different types of lithology (Figure S8), with the highest to lowest median Cr_T values observed in groundwater from the Floyd Church Formation, Cid Formation, mafic metavolcanic, phyllite and schist, biotite gneiss schist, felsic mica gneiss, felsic metamorphic, and granitic rocks, respectively (Table S2). The data show that groundwater from intermediate or mixed mafic metavolcanic formations has Cr_T concentrations (p < 0.05) significantly higher than those of groundwater from felsic formations (Table S3). These results are consistent with previous studies that have shown high Cr(VI) concentrations in groundwater from ultramafic rocks,[18−27] yet the data presented in this study infer Cr(VI) prevalence in groundwater from aquifers composed of metamorphic mafic rocks and even felsic rocks, which are highly common in the Piedmont region of the eastern United States.[42]

Environmental Health Implications. Assuming that Cr(VI) is the predominant Cr species in drinking water wells, we show that only 14 of 376 wells (∼4%) had Cr_T above the California MCL limit of 10 μg/L. At the same time, only 8 of 77 (∼10%) wells had Cr(VI) levels below the detection level (0.004 μg/L), meaning that 90% of the study wells had detectable Cr(VI), and furthermore, all of the detectable Cr(VI) was above the California PHG of 0.02 μg/L. While our sample collection was conducted in the Piedmont region of North Carolina, the distribution of the Piedmont rocks extends to other states in the eastern United States (see the top inset map in Figure 1), and a large population is potentially consuming drinking water with detectable and, in some cases, high Cr(VI) levels. Given the global distribution of aquifers composed of mafic and igneous rocks,[43] we hypothesize that

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the occurrence of Cr(VI) in shallow drinking water wells is much more widespread than previously thought, with possibly millions in the eastern United States and other parts of the world directly exposed to detectable Cr(VI) from drinking water wells. The lack of a national Cr(VI) standard for drinking water[4] impedes a large scale evaluation of the distribution of Cr(VI) in groundwater systems. Monitoring and screening for Cr(VI) levels in public and private wells are therefore essential for protecting human health in the Piedmont region and beyond.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.6b00342.

Eight figures, three tables, information about the analytical procedure of hexavalent chromium, and background on the hydrogeology of the Piedmont area (PDF)

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Notes

The authors declare no competing financial interest.

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The role of environmental buffers in potable water reuse

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The role of environmental buffers in potable water reuse

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1. Introduction

Estimated 14% of the US population lives in arid and semi-arid areas (US Census 2010). Those are also the areas experiencing the highest population growth according to the US Census data, with 5 arid states in the top 10 fastest growing states. Because of the insufficient water supplies to sustain the current and projected population, many of the arid areas practice or consider practicing potable water reuse. Apart from the acknowledged and purposefully implemented potable water reuse systems, there is a great number of instances where unacknowledged de facto potable water reuse is happening, i.e. when highly populated areas discharge treated effluent into the body of water that becomes a drinking water source for another downstream entity.

In most instances, water is released into an environmental buffer as it travels from a wastewater treatment plant (WWTP) to the downstream drinking water treatment plant (DWTP). Even in the instances of acknowledged water reuse, water is not pumped directly to the drinking water treatment plant but is rather allowed to percolate into an aquifer or to spend some time in a river or a reservoir. The reclaimed water that is destined for potable reuse applications is treated to the highest industry standards but subsequently is allowed to come in contact with various contaminants in the natural environment.

One of the main functions the environmental buffers serve is the improvement of public perception of water reuse, whether it is justified or not. The "yuck factor" is an important consideration in potable water reuse implementation projects (Schmidt 2008). Another potential benefit is environmental attenuation of contaminants via dilution, photolysis, hydrolysis, biodegradation and sorption. Some of the contaminants of concern in potable reuse water are pharmaceuticals and personal care products. While these contaminants are presently unregulated, multiple studies established their relevance to aquatic health. For example, chronic exposure to trace levels of pharmaceuticals have been demonstrated to cause disruption of predator avoidance patterns (Painter et al. 2009), feminization of male fish (Lange et al. 2008), and other endocrine disrupting effects (Conners et al. 2009). Apart from their relevance to environmental health, trace pharmaceuticals are linked to development of antibiotic resistance in the environment as a result of the contact between sub-inhibitory levels of antibiotics and microorganisms (Akiyama and Savin 2010, Goñi-Urriza et al. 2000). As a result, trace pharmaceuticals have direct relevance to human health. Other human health effects from chronic exposure to pharmaceutical mixtures in drinking water have been difficult to demonstrate and quantify, but are nevertheless possible (Pomati et al. 2006).

On the other hand, many of the contaminants, especially particulate matter and microorganisms can be reintroduced in the environmental buffer. As a result, the downstream DWTP requires treatment processes for removal of particulate matter and higher disinfectant levels which could be unnecessary if the DWTP were directly using treated wastewater effluent as source water. Environmental buffer can also introduce some of the unregulated emerging contaminants associated with urban and agricultural runoff, such as pesticides, herbicides and constituents of automotive fluids. In addition, trace levels of antibiotics discharged with treated wastewater get an opportunity to interact with microorganisms in the environment which could be one of the pathways of development of antibiotic resistance.

The main goal of this study is to answer the following questions: **Do environmental buffers mitigate contaminants or only public perception? Which contaminant classes get attenuated and which get introduced in the environmental buffer? How do specific types of environmental buffers (wetland, aquifer recharge, river, etc.) differ in that respect?** The

National Academy of Sciences assembled an expert panel on the water reuse topic in 2012, and one of the top seven research priorities identified by the panel for water reuse treatment efficiency and quality assurance was to develop a better understanding of contaminant attenuation in environmental buffers (National Academy of Sciences 2012).

The goal of this study is to measure the change in conventional water quality parameters as well as unregulated constituents of concern in several case studies representative of different types of environmental buffers. It will also estimate the costs to utilities for direct (pipe-to-pipe) vs. indirect (with environmental buffer) potable water reuse with each type of buffer involved.

If this study demonstrates that the environmental buffers serve only to recontaminate highly treated water and do little for attenuation of contaminants in most classes, the utilities armed with this information may ask an important question: Is it sensible to release highly treated water into the environment instead of taking it to the next level of engineered treatment? For example, water released into a river will require particle removal treatment at a DWTP. Instead, water that is already low in particulate matter coming from a WWTP could be treated by advanced treatment processes immediately. The cost saved on the particle removal and redisinfection could be applied to advanced treatment processes which could remove trace contaminants in a controlled and therefore more efficient manner than an environmental buffer could. While the answer to this question may appear obvious, no study currently exists that addresses this question in a systematic manner.

This study will produce materials that utilities will be able to use to communicate to their customers on the topic of water reuse, environmental buffers, and associated water quality. Communication materials that are accessible to a layperson but provide information with sufficient level of scientific detail can improve public trust in policy making and can open an avenue for informed public feedback (Veldhuis 2015).

2. Project goals and objectives

The main goal of this study is to answer the following questions:

- Do environmental buffers mitigate contaminants or only public perception?
- Which contaminant classes get attenuated and which get introduced in the environmental buffer?
- How do specific types of environmental buffers (wetland, aquifer recharge, river, etc.) differ in that respect?

To answer these questions, the following objectives were proposed:

1. Evaluate the ability of different types of environmental buffers (groundwater recharge, riverbank filtration, wetland treatment, and discharge into a river and a lake) to attenuate contaminants representative of different classes and different environmental fate. Specifically, compare water quality of the WWTP effluent to the water quality at the influent to the DWTP after it has passed through the environmental buffer (McDowell WWTP and Franklin DWTP, NC; Denver Metro and Englewood/Littleton WWTP and Aurora Prairie Waters, CO; Orange County Water District recharge and production well water, CA). Determine which classes of contaminants get attenuated and which get reintroduced in each type of environmental buffer (wetland, aquifer recharge, alluvial flow, river and lake). This objective includes analysis of conventional contaminants (suspended

solids, microorganisms, etc.) along with emerging contaminants (pharmaceuticals and antibiotic resistance genes - ARG).

2. Estimate the cost of existing potable water reuse systems if no environmental buffer was used. Based on the results of Objective 1, develop recommendations for utilities for potable water reuse. The recommendations would include the discussion of the treatment technologies appropriate for potable water reuse and the necessity (or lack thereof) for environmental buffers. Evaluate the cost and the logistical possibility of implementing the suggested recommendations.

3. Develop public communication materials for utilities based on the findings. Prepare a public education document/module to promote the optimal potable water reuse scenario based on the research results. The research results will be adapted to lay audience.

3. Activities

3.1. Summary

The project commenced in September 2016. The first few months of the project were allocated to purchasing supplies, developing methods, student training and site visits. During this time, the graduate student travelled to the USGS Kansas laboratory for one week to be trained on extraction and analysis methods for emerging contaminants. The student worked to develop sampling protocols and establish logistics with the utilities. The PI Olya Keen travelled to one of the collaborating utilities (Orange County Water District) to identify the appropriate sampling locations. Meetings were also conducted with Charlotte Water for the same purpose. The third site for this project is less challenging logistically, and decisions were arranged via email and phone conversations.

The timeline in the proposal allocated the bulk of time to Objective 1 as the most time consuming. The original goal was to complete this task within 18 months from the commencement of the project. The project is currently 8 month completed with active sampling going on for 4 months. To-date, of the 18 planned sampling events, 5 sampling events have been fully executed and the 6th is in process. The sampling is on schedule to be completed by the proposed deadline of March 2018.

3.2.Sampling sites

To-date, samples have been analyzed from two of the three participating locations, each exhibiting different environmental buffers or a combination thereof used in either acknowledged or de facto water resue.

Site 1: Orange County Water District (OCWD)
The site has groundwater recharge ponds that are supplied with water from two sources: (a) a constructed wetland that serves to purify river water (Santa Ana River) before it is routed to the groundwater recharge ponds; and (b) wastewater treatment plant effluent that went through advanced water purification system (AWPS) consisting of microfiltration, reverse osmosis and advanced oxidation. The water in Santa Ana River is largely impacted by effluent from two upstream wastewater treatment plants: San Bernardino and Riverside.

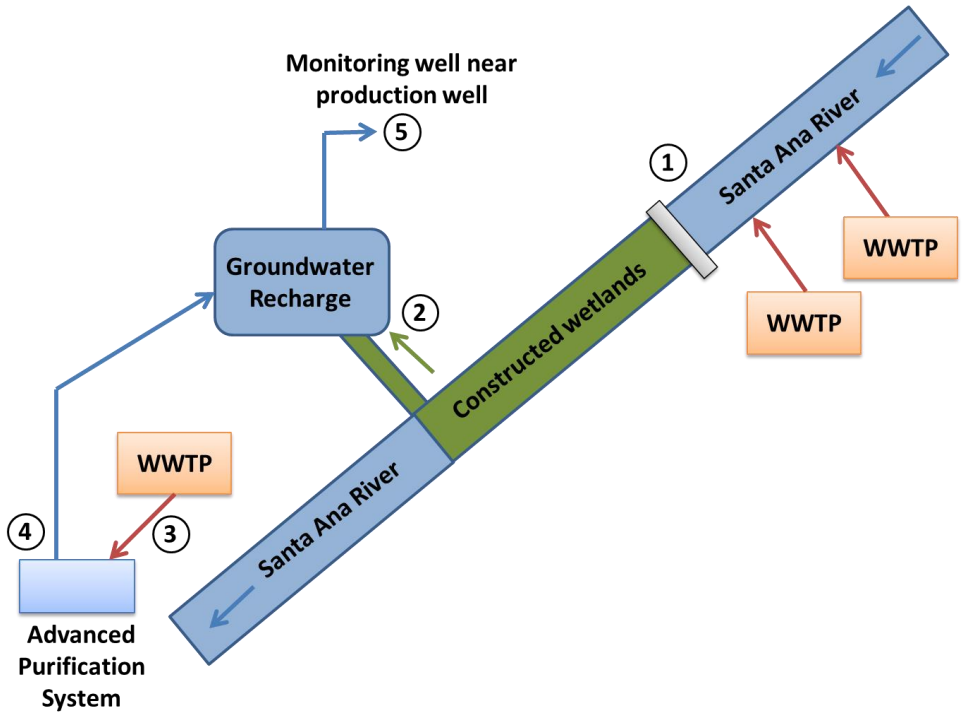

Figure 1: Sampling schematic for OCWD (It should be noted that a portion of Santa Ana River that is not used to feed the recharge basins bypasses the wetlands).

Site 2: Charlotte Water
Water from a local wastewater treatment facility flows with McDowell Creek into Catwaba River at the point where the river widens to form a lake (Mountain Island Lake). Water from Mountain Island Lake is used further downstream as a DWTP intake. Both Mountain Island Lake and Lake Norman located upstream are formed by dams on Catawba River and are located in highly urbanized areas.

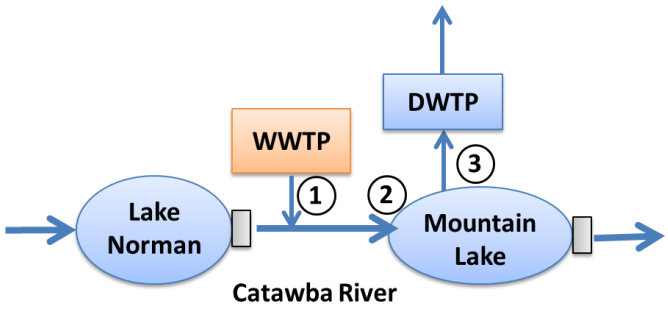

Figure 2: Schematics of Charlotte Water sampling locations

Site 3: Aurora Prairie Waters
The site uses South Platte River as a water source which largely consists of the effluent from several large wastewater treatment plants upstream: Englewood/Littleton and Denver Metro (two

collocated plants). The intake water is routed through a riverbank filtration system prior to advanced treatment that produces potable water delivered to utility customers.

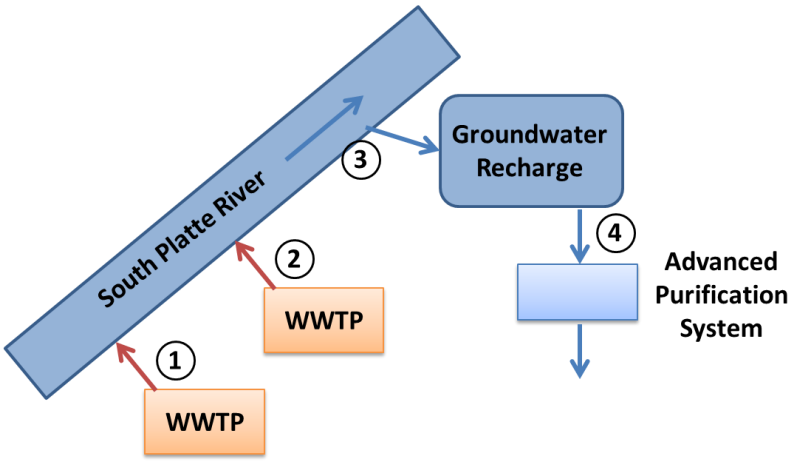

Figure 3: Sampling schematic for Aurora Prairie Waters

3.3. Sample collection, storage and processing

Participating utilities were supplied with coolers containing the description of the containers and the sampling technique. Containers were prepared per standard methods for each test and necessary preservatives were added as necessary (e.g. nitric acid for metals sampling). All samples were shipped with ice-packs to minimize exposure to heat, and maintained at 4 °C once they arrived at UNC Charlotte. Upon delivery, all sample containers were counted and verified according to the chain of custody forms. All broken or contaminated containers were recorded and valid substituting samples were used. Sample with 24 hour hold times were processed immediately, and remaining samples were processed before the corresponding hold time.

Biological samples (salmonella, coliform, E. coli, cryptosporidium, giardia and ARGs) are processed immediately. Salmonella, coliform, and E. coli enumeration is done using corresponding most probable number (MPN) methods and results can be obtained within 5 days. Cryptosporidium, giardia, and ARG enumeration results are obtained by using real-time polymerase chain reaction (qPCR). Samples are filtered using 0.45µm sterile nylon filters, vortexed in sterile phosphate dilution water (EPA Method 1680 2006), then centrifuged at 10,000rpm for 20min. The supernatant is then decanted and the concentrated filtered suspensions are currently stored at -80°C until further processing.

Metals and cations (B, Cd, Cu, Pb, Na, and Hg) are preserved in 2% nitric acid and stored in 4°C until further processing, currently scheduled to be analyzed on May, 10th 2017, using inductively coupled plasma optical emission spectrometry (ICP-OES). Anions (NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-}, Cl^- Br^-, and I^-) are analyzed using ion chromatography (IC) and HACH test kits. Due to unforeseen column contamination, samples collected after March 13th for Cl^-, Br^-, SO_4^{2-}, and I^- are still waiting to be analyzed. Replacement column is scheduled for delivery on May 15th.

Emerging contaminants (antibiotics: azithromycin, amoxicillin, cephalexin, ciprofloxacin, sulfamethoxazole-trimethoprim, doxycycline, levofloxacin, clindamycin, penicillin V; and

pharmaceuticals/pesticides: carbamazepine, sucralose, ibuprofen, glyphosate, and atrazine) will be analyzed using liquid chromatography-mass spectrometry and are extracted using solid phase extraction technique with hydrophilic-lipophilic-balanced (HLB) cartridges using the EPA method 1694 (Ferrer et al. 2010). Samples are eluted and stored at -20 °C in sterile glass vials. Samples are scheduled to be concentrated under nitrogen gas evaporation. Glyphosate is derivatized with HPLC grade 99% 9-fluorenylmethoxycarbonyl chloride (FMOC), and stored in 4°C waiting to be extracted (Lee et al. 2002). Benzo[a]pyrene will be analyzed using GC-FID following the EPA method 525.5.

Water characterization [total suspended solids (TSS), total organic carbon (TOC), pH, alkalinity, conductivity, 5 day biochemical oxygen demand (BOD_5), and chemical oxygen demand (COD)] are analyzed according to the EPA methods or Standard Methods (Rice et al. 2012). Nutrient analysis (total nitrogen and total phosphate) is done using HACH test kits. Results are all obtained prior to expiration of hold time.

Detailed methods and protocols are included in the Appendix 3, as well as a list of methods resources with links.

4. Findings and their significance

To-date, only conventional parameters have been analyzed on the collected samples. Samples for other parameters have been extracted and are preserved until more samples have been accumulated for a more efficient analysis.

Of the sites analyzed, the some trends can be remarked for various processes and are discussed in the sections below. These sections summarize and highlight the main observations with some of the more dramatic results shown in Figures 4 and 5. Tables containing results collected so far can be found in Appendix 4.

4.1. Attenuation in wetlands

Wetlands appear to be effective in decreasing the counts of wastewater indicator organisms (total coliforms, fecal coliforms, enterococci). Total coliforms were reduced by 94-99%, fecal coliforms by 91-95% and enterococci by > 98%. Salmonella counts did not show statistically significant change, but the counts in general were fairly low ranging from 0.7 to 8.8 MPN/100 mL. Wetland treatment also had a significant positive effect on lowering TSS by 96-97%, most likely due to slowing of the flow as the stream entered the wetlands, which allowed particulate matter to settle. Some of the reduction of microbial counts could be associated with the settling of particulates as well. The following parameters showed no observable change in wetland treatment based on the two samples collected to-date: TOC, BOD_5, conductivity, pH, alkalinity, and COD. There appears to be some incomplete denitrification as nitrite levels increase significantly (from 0.025 mg/L to 0.20 mg/L as N) while nitrate is lowered (from 4.5 mg/L to 1.1 mg/L as N). These values are averages of two samples. The increase in nitrite level is not high enough to cause a concern based on these observations (high value was 0.35 mg/L as N). Total nitrogen was lowered in wetland treatment by 30-70% while no significant decrease in total phosphorus was observed. The parameters that were negatively affected by wetland treatment were chloride and sulfate concentrations (increased from 20 mg/L to 148 mg/L and from 35

mg/L to 72 mg/L respectively). Anions were analyzed on one of the two samples, and it remains to be seen whether this trend is consistent.

4.2. Attenuation through advanced water purification

As expected, all parameters were majorly improved by advanced water purification. Microbial counts were all below detection level after treatment with very high levels in the influent (>2420 MPN/100 mL of total coliforms, fecal coliforms and enterococci, and 9-27 MPN/100 mL of Salmonella). TOC was reduced by 99% to 0.13 ± 0.01 mg/L, chloride was reduced by 98% to 4.5 mg/L, bromide by 79% to 0.02 mg/L, sulfate by 99% to 1.6 mg/L, BOD_5 by 98% to 0.3 mg/L to below detection limit, TSS by 92% to 0.2-0.8 mg/L, conductivity by 98% to 35 ± 9 mg/L, alkalinity by 96% to 5 ± 2 mg/L as $CaCO_3$, nitrate by 98% to 1.0 ± 0.0 mg/L as N, total phosphorus by 96% to 0.08 ± 0.03 mg/L, and COD by 90% to 4-15 mg/L. Results for samples with high consistency between the two sampling events are reported as an average with standard deviation margins, and the values showing larger range are shown as a range with the two measurements as the upper and lower end. pH lowered in the process from 7.2 ± 0.0 to 6.0 ± 0.4. The water was collected prior to remineralization, and the pH of the finished water is raised closer to the influent pH after the sampling point. No iodide was detected in any samples, and nitrite was below detection limit (BDL) for all samples except one influent sample where nitrate was 0.215 mg/L as N. Phosphate was extremely low in the influent (0.02-0.04 mg/L) and was reduced further by approximately 70%.

4.3. Attenuation in groundwater recharge system

The groundwater recharge system is fed by recharge ponds that contain the water treated through wetlands and water from AWPS. Wetland treated water contained fairly high counts of bacteria. However, no tested organisms were detected in the monitoring well near the production well. The production well quality was close to that of AWPS in terms of TOC, Cl^-, SO_4^{2-}, BOD_5, TSS, conductivity. Slight increase in nitrate, bromide and alkalinity was observed. Alkalinity increase is a natural phenomenon and is most likely the result of dissolution of minerals during recharge. Nitrate and bromide, on the other hand, most likely come from non-point sources in the surrounding area and are the result of human activity. The concentrations of both remained very low and well below any levels of concern (1.5 mg/L as N for nitrate and 0.07 mg/L for Br^-), although they demonstrate the potential for contamination of groundwater in aquifer recharge systems. Total phosphorus was the only parameter that was much higher in well water than in AWPS water, but still lower than in wetland effluent (Figure 4A). For comparison, chloride in the wetlands effluent, AWPS effluent and groundwater recharge monitoring well is also shown (Figure 4B) to indicate that the increase in total phosphorus cannot be attributed to the wetlands effluent and most likely enters groundwater from non-point sources. Phosphate remains low in all samples (wetland effluent, AWPS effluent and monitoring well), therefore the increase in total phosphorus can potentially be attributed to organic compounds containing phosphorus. While many of those are benign, samples will be analyzed for presence of organophosphate pesticides. Overall, groundwater recharge appeared to provide more benefit than risk based on the two samples and on the conventional parameters analyzed so far. It appears to provide a low-cost treatment to an impaired source (wetlands effluent) and it does not appear to significantly contaminate the highly purified reclaimed water.

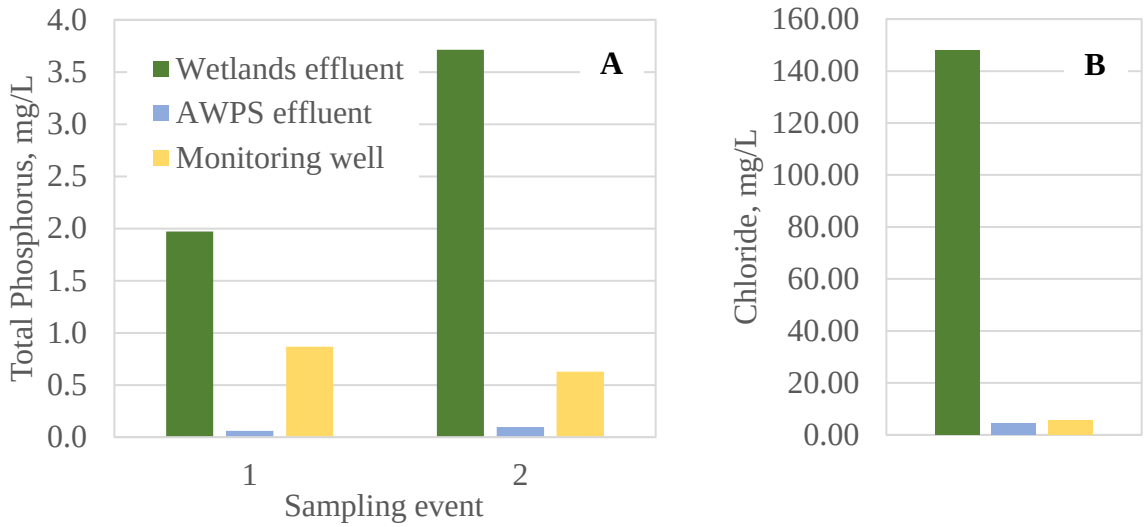

Figure 4: Total phosphorus (A) and chloride (B) concentration in groundwater recharge system monitoring well and in two sources used for groundwater recharge: wetlands-treated stream water consisting primarily of wastewater effluent and advanced water purification system (AWPS) effluent

4.4. Attenuation in a lake system

In general, lake water had good microbial quality with 1-3 MPN/100 mL of total coliforms, 0-3 MPN/mL of fecal coliforms, 0 MPN/100 mL of enterococci and 0.1-0.65 MPN/100 mL of Salmonella during the two dry weather sampling events. For the third sample that was collected after substantial rainfall, much higher levels of tested microorganisms were observed. Additionally, microorganism counts increased from the point where wastewater effluent mixed with the lake to the downstream point where drinking water intake is located (Figure 5). The increase in microorganisms is likely related to non-point sources (e.g. runoff that may contain animal excrement). Wastewater effluent microbial quality was not affected by increased rainfall, although the flow through the wastewater treatment plant and the need for temporary storage in equalization basins increased. To-date, only one wet weather sample of the three planned was collected, and future data will reveal whether the observations are trends for wet weather. The lake system in general appears to provide a substantial dilution for a number of parameters: TOC, alkalinity, conductivity, nitrate and COD. TSS was comparable in effluent and in lake water. Nitrite was below detection limit for all samples. There was some attenuation of total nitrogen and total phosphorus in the lake system.

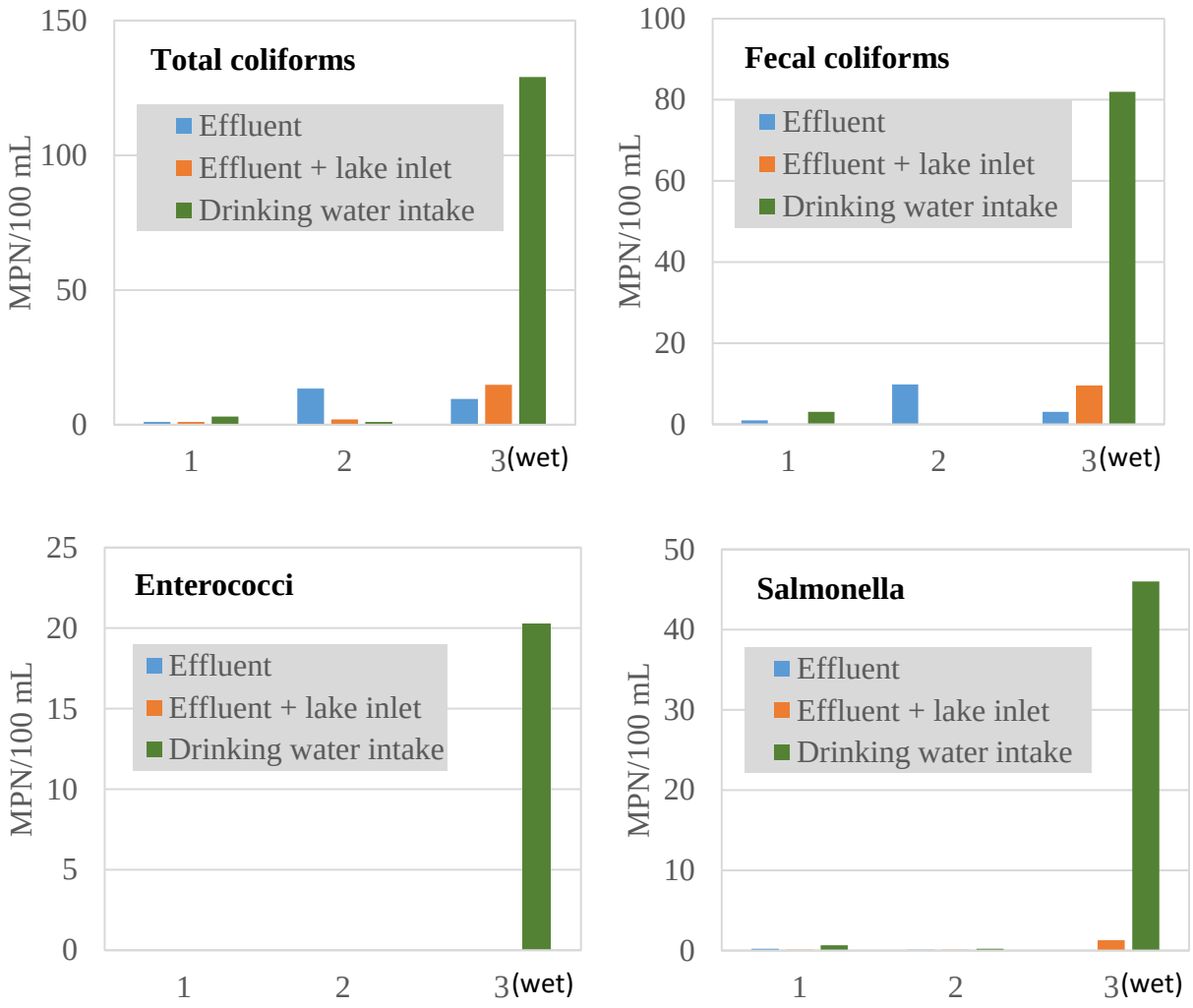

Figure 5: Effect of wet weather on microbial quality of the lake environmental buffer. Microbial counts for three sampling events, one of which was impacted by wet weather.

4.5. Next steps

Sampling is currently underway at the third location in this study: river attenuation and riverbank filtration site. Sampling is scheduled to go on through March of 2018 and is currently ~30% complete. In September-October of 2017, it is anticipated that enough data will be collected to begin an economic and regulatory analysis of the use of environmental buffers vs. direct potable reuse. In August-September, the first batch of extracted samples will be analyzed on HPLC-MS and GC for organic contaminants of interest to this study. In February-March, as sampling and analysis nears the end, participating utilities will be contacted regarding developing a public communication message/module to disseminate the results of the study.

4.6. Significance of findings

Although the original hypothesis was that the environmental buffers mainly serve to mitigate the public perception of water reuse, limited data collected so far suggest that at least from the perspective of conventional water parameters, environmental buffers can be of value or at least

of no harm. Currently it appears that wetlands can be effective for mitigating microbial contamination and TSS of an otherwise impaired water source with influent microbial counts of ≥ 550 MPN/100 mL and TSS of up to 293 mg/L, and can provide some marginal reduction in total nitrogen. It must be noted that both of the wetland samples analyzed to-date are wet weather samples, and it is possible that the microbial contaminants and the TSS levels in the influent are lower in dry weather and that the effect of the wetlands on improving those parameters is much less pronounced. Groundwater recharge system was highly effective in removing microbial contaminants, TSS, TOC and in general had levels of all measured conventional parameters close to those of highly purified water. The lake system in this study provided an effective attenuation by dilution, with many conventional water quality parameters in the lake water better than in treated wastewater effluent. However, it wet weather when runoff volumes were high, the lake was susceptible to microbial contamination that was not observed in dry weather.

5. Student involvement

The project involves a graduate and an undergraduate student. The graduate student Xueying Wang has worked to develop the methods, the sampling schedule, has been coordinating with the sampling location contacts and handling the more complex analysis: microbial testes, sample extractions, tests requiring the use of complex instruments, e.g. IC, ICP-OES, GC and HPLC/MS. She has travelled to the USGS laboratory in Kansas to be trained on trace organics extraction and analysis. Additionally, an abstract was submitted to AWWA International Symposium on Potable Reuse, which will take place January 22-23, 2018 in Austin, TX. The presentation will be given by Xueying who is the first author on the abstract.

The undergraduate student, Brittany Hause, has worked under Xueying's supervision. Her tasks are to measure routine water quality parameters (BOD_5, TSS, pH, alkalinity, etc.) including all relevant QA/QC.

6. Deviations from original project plans

Any changes to the project are minor and are not expected to affect the ability to address the project objectives. Challenges encountered so far are below:

a. Sampling of the Charlotte location where the effluent from the wastewater treatment plant mixes with the lake inlet turned out to be inaccessible from the shore due to a steep drop at the available non-private-property location that was previous considered. A canoe was purchased, and the students travel approximately 1.5 mi by canoe to access the intended location from water.
b. The Denver Metro wastewater treatment plant consists of two separate plants, and the operators did not consider it possible to collect a mixed sample based on the sampling protocols. Therefore, two separate samples will be collected from that location and analyzed, rather than one mixed sample as originally planned. It currently appears that the budget is sufficient to accommodate this additional sampling location.

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Index of Appendices

Appendix 1: Abbreviations and symbols

Abbreviations:

ARG = antibiotic resistance genes
AWPS = advanced water purification system
BDL = below detection limit
BOD_5 = 5-day biochemical oxygen demand
COD = chemical oxygen demand
DWTP = drinking water treatment plant
FMOC = 9-fluorenylmethoxycarbonyl chloride
GC = gas chromatography
GC-FID = gas chromatography - flame ionization detector
HLB = hydrophilic-lipophilic balanced
HPLC = high performance liquid chromatography
HPLC-MS = high performance liquid chromatography - mass spectrometry
IC = ion chromatography
ICP-OES = inductively coupled plasma - optical emission spectrometry
MPN = most probable number
OCWD = Orange County Water District
QA/QC = quality assurance/quality control
qPCR = real-time (quantitative) plymerase chain reaction
TOC = total organic carbon
TSS = total suspended solids
WWTP = wastewater treatment plant

Symbols:

B = boron
Br^- = bromide
$CaCO_3$ = calcium carbonate
Cd = cadmium
Cl^- = chloride
Cu = copper
Hg = mercury
I^- = iodide
N = nitrogen
Na = sodium
NO_2^- = nitrite
NO_3^- = nitrate
Pb = lead
PO_4^{3-} = phosphate
SO_4^{2-} = sulfate

Appendix 2: Results dissemination, research products

As the project is currently only approximately 30% complete, the results collected are not ready for dissemination. An abstract was submitted in April to AWWA International Symposium on Potable Reuse, which will take place January 22-23, 2018 in Austin, TX. Much of the data is expected to be collected and processed by January. After the conference, the research team will work on publishing the results.

Appendix 3: Analytical protocols

Microbiology

Salmonella

Membrane Filtration for Salmonella Concentration (9260B-1d)

1. Low turbidity water – Filter several liters (note the amount used) through a sterile 142-mm (0.45 µm) membrane filter

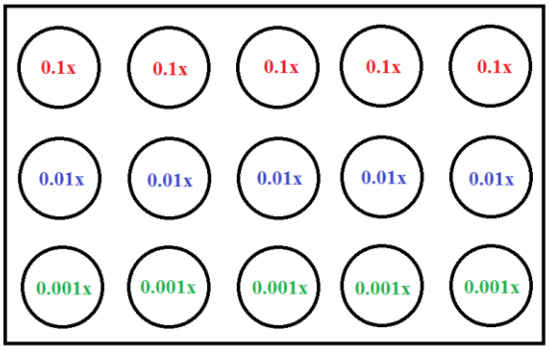
   High turbidity water – Precoat sterile 142-mm (0.45 µm) membrane filter with 500 mL of diatomaceous earth suspension (aids filtration).
2. Immediately add desired sample water volume to the filter without interrupting filtration.
3. Place filtered membrane in a sterile bender jar containing 100 mL sterile peptone water and homogenize at high speed for 1 min.

Microbial Enrichment for MPN Method (9260D)

4. Make serial dilution of the sample homogenate with double strength selenite cysteine broth (0.1x, 0.01x, and 0.001x) total volume 50 mL.

Dilution	Concertation of stock sample homogenate/selenite cysteine broth (mL/100 mL)	Volume of stock homogenate added (mL)	Volume of selenite cysteine broth added (mL)	Final concentration of sample homogenate to selenite cysteine broth (mL/L)
0.1x	n/a	2.5 mL	22.5 mL	0.1
0.01x	10/100	2.5 mL	22.5 mL	0.01
0.001x	1/100	2.5 mL	22.5 mL	0.001

5. Proportion sample homogenate into a five-tube, three-dilution multiple-tube procedure in double strength selenite cysteine broth (9221C and 9260B 2-a). Perform in 50 mL centrifuge vials.



6. Incubate MPN glass vials for 48 h at 35-37 °C (time and temperature specific to selenite cysteine broth enrichment).
7. After incubation, using sterile inoculation loop, streak from each MPN vial to individual plates of brilliant green and xylose lysine desoxycholate agars. Incubate upside down (to prevent condensation from falling onto the plates) for 24 hours at 35°C

8. Select from plate at least 1 (preferably 2-3) salmonella colonies, using sterile cell scraper, inoculate a triple sugar iron and lysine iron agar slant tube*. Look for white or opaque black bacterial colonies.
    a. Sterilize the inoculating needle in the blue flame of the Bunsen burner till red hot and then allowed to cool.
    b. Take a sterile TSI or LIA slant tube from the rack, remove the cap and flame the neck of the tube.
    c. Stab the needle containing the pure culture into the medium, upto the butt of the TSI/LIA tube, and then streak the needle back and forth along the surface of the slant.
    d. Again flame the neck of the TSI/LIA tube, cap it and place it in the test tube rack.
        i. TSI slant (Salmonella produces alkaline red slants and acid yellow butt with/without gas bubbles, and blackening). Salmonella is a non lactose fermenter thus have pink slant and yellow butt
        ii. LIA slant (salmonella produces black butt with red slant)
9. Estimate Bacterial Density using most probable number (MPN) (EPA Table 9221:IV and 9221C)

<u>Total and Fecal Coliforms and Enterococci</u>

Total coliform and E. coli (fecal coliform)

The method Colilert-18/Quanti-Tray or Quanti-Tray/2000 for water analysis is granted NF Validation by AFNOR Certification as an alternative method to the standard ISO 9308-3 for enumeration of Escherichia coli ß-glucuronidase positive in bathing water, under the Certificate number: IDX 33/02–06/12.

Quanti-Tray Enumeration Procedure (including Absence/Presence)

1. Place 100mL of sample in a sterile mL IDEXX vessel (with sodium thiosulfate).
2. Add the contents of one pack of colilert reagent to the vessel.
3. Cap vessel and shake until thoroughly dissolved.
4. Pour sample/reagent mix into a Quanti-Tray/2000
5. Seal the tray with the IDEXX Quanti-Tray Sealer.
6. Place the sealed tray in a 35 ± 5 °C incubator for 18-22 hours (at the researcher's convenience).
7. Sample observation conditions and interpretation using Table 1. below
    a. Compare incubated samples to the comparator in normal lighting conditions for total coliforms
    b. Compare sample under 6 watt 365 nm UV light in darkened environment with comparator for E. coli.
8. Using tables provided by IDEXX obtain the most probable number for total coliform and E. coli.

Quality Control/Quality Assessment

1. Repeat above steps with 100 mL of sterile ultrapure water
2. Repeat above steps with active E. coli cultures

Table A3-1. Results interpretation of presence/absence procedure and Quanti-Tray enumeration procedure.

Appearance of Vessel	**Result**
Less yellow than the comparator1 when incubated at 35 ± 0.5 °C or 44.5 ± 0.2 °C	Negative for total coliforms and E. coli; Negative for fecal coliforms
Yellow equal to or greater than the comparator when incubated at 35 ± 0.5 °C	Positive for total coliforms
Yellow equal to or greater than the comparator when incubated at 44.5 ± 0.2 °C	Positive for fecal coliforms
Yellow and fluorescence equal to or greater than the comparator when incubated at 35 ± 0.5 °C	Positive for E. coli

Total Enterococci

Enterolert detects enterococci, such as E. faecium and E. faecalis, in fresh and marine water. It is based on IDEXX's patented Defined Substrate Technology (DST). When enterococci utilize their ß-glucosidase enzyme to metabolize Enterolert's nutrient-indicator, 4-methyl-umbelliferyl ß-D-glucoside, the sample fluoresces. Enterolert detects enterococci at 1 CFU per 100 mL sample within 24 hours.

Quanti-Tray Enumeration Procedure (including Absence/Presence)

1. Place 100 mL of sample in a sterile IDEXX vessel (with sodium thiosulfate).
2. Add the contents of one pack of enterolert reagent to the vessel.
3. Cap vessel and shake until thoroughly dissolved.
4. Pour sample/reagent mix into a Quanti-Tray/2000
5. Seal the tray with the IDEXX Quanti-Tray Sealer.
6. Place the sealed tray in a 41 ± 0.5 °C incubator for 24 hours
7. Sample observation conditions and interpretation using Table 2
    a. Observe sample under 6 watt 365nm UV light in darkened environment to check for fluorescence.
8. Using tables provided by IDEXX obtain the most probable number for enterococci

Quality Control/Quality Assessment

1. Repeat above steps with 100 mL of sterile ultrapure water
2. Repeat above steps with active E. faecalis cultures

Table A3-2. Results interpretation of presence/absence procedure and Quanti-Tray enumeration procedure.

Appearance of Vessel	**Result**
Lack of fluorescence	Negative for enterococci
Blue fluorescence	Positive for enterococci

Real-time polymerase chain reaction (qPCR)

PCR sample filtration/concentration for cryptosporidium/giardia and antibiotic resistant genes

Filtration Method

Blank

1. Set up sterile filter apparatus
    a. Sterilize forceps with flame
    b. Using forceps, place sterile membrane filter on mesh lined side up
2. Filter 1 L of 18 MΩ – ultrapure water (or note volume used)
3. Place filter in the 50 mL centrifuge tube containing 30 mL of buffer solution.
    a. Sterilize forceps and gently pick up the used filter.
    b. Carefully roll the filter (lined side in) and place it in the centrifuge tube.
    c. Take care not to cross contaminate.

Sample

1. Use the same filter apparatus as the blank (without re-sterilization)
    a. Sterilize forceps with flame
    b. Using forceps, place sterile membrane filter on mesh lined side up
2. Filter sample (note volume used)
    a. If sample is turbid, more than one filter can be used.
3. Place filter in the 50 mL centrifuge tube containing 30 mL of buffer solution.
    a. Sterilize forceps and gently pick up the used filter.
    b. Carefully roll the filter (lined side in) and place it in the centrifuge tube.
    c. Take care not to cross contaminate.

Concentration Method

1. Vortex centrifuge tube containing the filter for a minimum of 2 minutes.
    a. Vortex in short intervals as careful not to tear the filters.
2. Remove filter using sterilized forceps
    a. If pieces break off, remove all pieces with sterilized inoculation loop
3. Centrifuge the solution
    a. 10000 rpm for 20 minutes
    b. rotate the tube and centrifuge again for 3 minutes at 5000 rpm
4. Decant the supernatant with a sterile pipette until 4mL of the solution remains
    a. DO NOT DISTURBE THE PELLET COLLETED AT THE BOTTOM
5. Mix the pellet with the remaining 4mL of buffer solution using a flamed-sterile loop until completely dissolved/homogenized.
6. Transfer the homogenized concentrate to a cryogenic tube using a sterile pipette and store at -80°C.

Nutrients

Chemical Oxygen Demand (HACH/EPA Method 8000)

1. Homogenize samples by shaking the sample container for 30 seconds.
2. Set the DRB200 Reactor power to on. Preheat to 150 °C.
3. Remove the cap from a vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of sample to the vial.
4. Remove the cap from a second vial for the selected range. Hold the vial at an angle of 45 degrees. Use a clean pipet to add 2.00 mL of deionized/ultrapure water to the vial.
5. Close the vials tightly. Rinse the vials with water and wipe with a clean paper towel.
6. Hold the vials by the cap, over a sink. Invert gently several times to mix.
7. Put the vials in the preheated DRB200 reactor. Close the lid and heat the vials for 2 hours.
8. Set the reactor power to off. Let the vials cool in the reactor for approximately 20 minutes to 120 °C or less.
9. Invert each vial several times while it is still warm.
10. Put the vials in a tube rack to cool to room temperature.
11. Start program 431 COD ULR, 430 COD LR or 435 COD HR.
12. Clean the blank sample cell.
13. Insert the blank into the cell holder. Push ZERO. The display shows 0 or 0.0 mg/L COD. Clean the prepared sample cell. Insert the prepared sample into the cell holder.
14. Push READ. Results show in mg/L COD.

Total Nitrogen (HACH/EPA Method 10072)

1. Start the DRB200 reactor. Set the temperature to 105 °C.
2. Use a funnel to add the contents of one Total Nitrogen Persulfate Reagent Powder Pillow to each of two HR Total Nitrogen Hydroxide Digestion Reagent vials. Make sure to clean any reagent that gets on the lip of the vials or on the vial threads.
3. Add 0.5 mL of sample to one of the vials.
4. Add 0.5 mL of ultrapure water to the second vial.
5. Put the caps on both vials. Shake vigorously for at least 30 seconds to mix. Undissolved powder will not affect the accuracy of the test.
6. Put the vials in the reactor and close the lid. Leave the vials in the reactor for exactly 30 minutes.
7. At 30 minutes, use finger cots to immediately remove the vials from the reactor. Let the vials cool to room temperature.
8. Start program 394 N, Total HR TNT.
9. Add the contents of one Total Nitrogen (TN) Reagent A Powder Pillow to each vial.
10. Put the caps on both vials. Shake for 30 seconds.
11. Start the instrument timer. A 3-minute reaction time starts.
12. After the timer expires, remove the caps from the vials. Add one TN Reagent B Powder Pillow to each vial.

13. Put the caps on both vials. Shake vigorously for 15 seconds to mix. The reagent will not dissolve completely. Undissolved powder will not affect the accuracy of the test. The solution will start to turn yellow.
14. Start the instrument timer. A 2-minute reaction time starts.
15. When the timer expires, use a pipet to put 2 mL of the digested, treated prepared sample/blank into one TN Reagent C vial.
16. Put the caps on both vials. Invert 10 times to mix. Use slow, deliberation inversions for complete recovery. The vials will be warm to the touch.
17. Start the instrument timer. A 5-minute. Reaction time starts. The yellow color will intensify.
18. When the timer expires, clean the blank vial.
19. Insert the blank vial into the 16-mm cell holder.
20. Push ZERO. The display shows 0 mg/L N. Clean the sample vial. Insert the sample vial into the 16-mm cell holder.
21. Push READ. Results show in mg/L N.

Total Phosphorus (HACH/EPA Method 8190)

1. Start the DRB200 Reactor. Preheat to 150 °C.
2. Start program 536 P Total/AH PV TNT.
3. Add 5.0 mL of sample to the Total Phosphorus Test Vial.
4. Add the contents of one Potassium Persulfate Powder Pillow for Phosphonate to the vial.
5. Put the cap on the vial. Shake to dissolve the powder. Insert the vial into the reactor. Close the reactor.
6. Start the instrument timer. A 30-minute reaction time starts.
7. When the timer expires, carefully remove the vial from the reactor. Set the vial in a test tube rack. Let the vial cool to room temperature.
8. Add 2 mL of 1.54 N Sodium Hydroxide Standard Solution to the vial.
9. Put the cap on the vial. Invert to mix. Clean the vial. Insert the vial into the 16-mm cell holder. Push ZERO. The display shows 0.00 mg/L PO_4^{3-}.
10. Add the contents of one PhosVer 3 Powder Pillow to the vial.
11. Put the cap on the vial. Shake to mix for 20–30 seconds. The powder will not dissolve completely. Start the instrument timer. A 2-minute reaction time starts. Measure the sample within two to eight minutes after the timer expires.
12. Clean the vial. Insert the vial into the 16-mm cell holder. Push READ. Results show in mg/L PO_4^{3-}.

5-Day Biochemical Oxygen Demand (EPA Method 5210 B)

Preliminary Work

1. Autoclave all glass BOD bottles and stoppers.
2. Prepare dilution water
    a. Obtain appropriate volume of DI water in polypropylene container(s) 2 days prior to sample analysis.
    b. Autoclave the DI water.

c. Add buffer nutrients immediately prior to sample analysis to prevent unwanted microbial growth.
d. Prior to sample dilution
    i. Shake the dilution water container prior to sample analysis to ensure dissolved oxygen saturation.
    ii. Check to ensure the dilution water DO level is at least 7.5 mg/L.
    iii. If DO level is less than 7.5 mg/L, continue to aerate and shake container until the desired DO level is reached.

3. Calibrate DO meter per instructions on the back.

Sample Analysis

1. Adjust sample temperature to 20 ± 3°C
2. Check sample pH
    a. If pH is not between 6.0-8.0, adjust pH to 7.0 – 7.2 using sulfuric acid or sodium hydroxide.
3. Make twin dilution bottles

3 dilutions bottles and one quality control

Bottle #	Dilution water volume (mL)	Sample water volume (mL)
1, 4	50	250
2, 5	150	150
3, 6	200	100
4*, 7*	300	0

*Quality control

4. After dilution, measure the DO of one set of twin bottles and record it.
5. Stopper and parafilm the other set of twin bottles
    a. Place bottles in a dark environment to prevent photosynthetic growth
    b. Incubate bottles at temperature between 20 ± 3°C
6. After 5 days, measure the DO of the incubated bottles and calculate BOD using the formula:

$$BOD_{5\ (mg/L)} = \frac{(Inital\ DO - Final\ DO)}{(\frac{(mL\ of\ sample)}{300\ mL})}$$

QA/QC

1. Sample bottles should have a minimum DO depletion of 2.0 mg/L and a residual DO of 1.0 mg/L
2. The control dilution water should not have a DO depletion of more than 0.20 mg/L

Anions

Total Phosphate (HACH/EPA Method 8048)

1. Start program 535 P React. PV TNT.
2. Add 5.0 mL of sample to a Reactive Phosphorus Test 'N Tube Vial. Put the cap on the vial. Invert to mix.
3. Clean the vial. Insert the vial into the 16-mm cell holder. Push ZERO. The display shows 0.00 mg/L PO_4^{3-}.
4. Add the contents of one PhosVer 3 Phosphate Powder Pillow. Put the cap on the vial. Shake for at least 20 seconds. The powder will not dissolve completely.
5. Start the instrument timer. A 2-minute reaction time starts. When the timer expires, clean the vial.
6. Insert the vial into the 16-mm cell holder. Push READ. Results show in mg/L PO_4^{3-}.

Total Nitrate (HACH/EPA Method 10206)

1. Use a pipet to add 1.0 mL of sample/blank to the test vial. Use a pipet to add 0.2 mL of Solution A to the test vial.
2. Tighten the cap on the vial and invert until completely mixed.
3. Start the reaction time of 15 minutes. When the timer expires, clean the vial.
4. Using DR 1900 only: Select program 835.
5. Insert the vial into the cell holder. DR 1900 only: Push READ. Results show in mg/L NO_3^-–N.

Total Nitrite (HACH/EPA Method 10237)

1. Carefully remove the lid from the DosiCap™ Zip cap. Remove the cap from the test vial.
2. Use a pipet to add 0.2mL of sample to the test vial. Immediately continue to the next step.
3. Turn the DosiCap Zip over the test vial so that the reagent side goes on the vial. Tighten the cap on the vial.
4. Shake the vial 2–3 times to dissolve the reagent in the cap. Look through the open end of the DosiCap to make sure that the reagent has dissolved.
5. Start the reaction time of 10 minutes. When the timer expires, clean the vial. DR 1900 only: Select program 840.
6. Insert the vial into the cell holder. DR 1900 only: Push READ. Results show in mg/L NO_2^-–N.

Sulfate, chloride, iodide, bromide (Ion Chromatography)

1. Filter 40 mL of sample through 0.45 µm glass fiber filter.
2. Transfer sample to 10 mL IC sample vials.
3. Run sequence for EVERY SITE (even if multiple sites are run within the same day):
    a. 1 blank (ultrapure water)

    b. 1 standard (25 mg/L (I^-, Cl^-, SO_4^{2-}) and 2.5 mg/L (Br^-)) at the beginning and every 10 samples.
        i. 2 mL of 100 mg/L stock + 6mL of ultrapure water = 25 mg/L (8 mL total)

c. Spike samples for every sample site (3 samples + 1 sample with spike, 4 samples per site total).
    i. Added spike concentration 12.5 mg/L
    ii. Add 0.1 mL of 1000 mg/L stock (0.1mL from each original stock solution, (I^-, Cl^-, and SO_4^{2-}) and 0.1 mL of 100 mg/L Br^- stock to 7.6 mL of sample water (8mL total)

(Blank – Standard – Sample 1 – Sample 2 – Sample 3 – Spiked Sample)

Eluent solution

0.9539 g Na_2CO_3 + 0.2352 g $NaHCO_3$ + 2 L ultrapure water

Metals

Inductively coupled plasma atomic emission spectroscopy

Boron, copper, sodium, lead, cadmium, and mercury

Sample Preparation and Storage

1. Samples are collected in metal free, nitric acid rinsed polypropylene plastic bottles.
2. Samples are preserved in nitric acid and stored in 4 °C until analysis.

Emerging Contaminants

Liquid Chromatography/Mass Spectrometry

Pesticides, pharmaceuticals, and antibiotics

Preliminary Work

1. Obtain 1 mL sample weight.
    a. Weigh empty glass vial and record weight (W1).
    b. Add 50/50 1mL methanol and acetonitrile and weight test tube, record the weight (W2).
    c. (W2) – (W1) = weight of 1 mL of solution.
    d. Or use tare for the weight of 1mL solution
2. Adjust pH to ≤ 2
3. Filter sample through 0.2-0.8 μm glass fiber filter.
4. Rinse all glassware (including glass test tube) 3 times with HPLC grade water.
5. Add deuterated carbamazepine-D_{10} to sample water for loss recovery.
    a. Stock solution = 1 μg/mL
    b. Per 1 L of filtered sample, add 1mL of the 1 μg/mL solution.
        i. 1 μg of carbamazepine-D_{10} per liter of sample.

Solid Phase Extraction

1. Wash the HLB cartridge with 5 mL of methanol.
2. Condition the cartridge with 5 mL of solvent grade water.
3. Elute the sample through the cartridge.
    a. Note the start and end time for rate calculation.
4. Elute the sample cartridge with 2.5 mL methanol then 2.5 mL acetonitrile.

5. Evaporate the test tube using nitrogen evaporator until < 1mL remains
6. Using the weighing method, reconstitute the sample using 50/50 methanol and acetonitrile until the weigh reaches previously recorded for the 1 mL solution.
7. Transfer sample to HPLC vial and store in -20 °C indefinitely.

Glyphosate

Sample Preparation

Sample should be derivatized within 5 days and stored in 4 °C in dark

1. Filter sample water
2. Dispense 10 mL of sample into plastic vials.
3. Add 200 µL of the 50 µg/L working standard to each of the 10 mL samples (per 10 mL sample will have the concentration of 1 µg/L of the isotope)
    a. Do QA/QC every 10 samples (SPE per batch so add internal standard to batch)
    b. Internal standard calculation
        i. Internal standard stock made at 100 µg/L
        ii. Add 100 µL to make up to 10 mL of sample
        0.1 mL of 100 µg/L glyphosate-D_3 stock + 9.9 mL of sample

4. Add 0.5mL of 5% sodium borate solution.
5. Mix the solutions in the tubes (vortex is recommended).
6. Add 1.5 mL of 2.5 mM FMOC in acetonitrile.
7. Invert 3 times to mix
8. Place all tubes in 40 °C water bath in the dark for incubation for 24 ± 1 hours.
9. After incubation add 0.6 mL of 2% phosphoric acid in HPLC grade ultrapure water to the tubes.
10. Inver 3 times to mix
11. Store derivatized samples in the dark at 4 °C until analysis.

Solid Phase Extraction

1. Condition HLB cartridge sequentially with:
    a. 2 mL of methanol
    b. 2 mL of DI water
2. Load the 10 mL of derivatized samples.
3. Wash cartridge with 1 mL of DI water
4. Elute with 5 mL of 50/50 ammonia acetate in HPLC grade water and ACN
5. Evaporate until 1mL of below 1mL reconstitute with 50/50 mixture.
6. Store elution in -20 °C indefinitely.

Gas Chromatography (EPA Method 6440B-3)

Benzo[a]pyrene

Sample Collection and Storage (EPA-Method 525.2-8.0)

1. Sample Collection
    a. Collect samples in 1L (Teflon lined cap screws) ashed amber glass bottles
    b. Sampling equipment must be free of plastic tubing, gaskets and other parts that may leach interfering analytes into water sample.
    c. Add 40-50 mg of sodium sulfate to each sample, stir/shake until dissolved (to reduce chlorine).
    d. Add 6 N HCl to sample until pH is < 2 (to reduce microbial degradation of analyte)
    e. Keep container sealed until extraction/analysis
2. Sample Preservation and Hold Time
    a. All samples are iced/refrigerated at 4 °C away from light
    b. Samples must be extracted within 14 days (stored at 4 °C away from light)
    c. Extracts must be analyzed within 30 days after extraction.

Solid Phase Extraction

Preliminary Work

1) Wash all glassware in dish washer then rinse 3 times with ultrapure water.
2) Obtain glass tube and mark 6 mL and 10 mL volume line.
3) SPE cartridge: Bond-Elut 500 mg

Extraction Method

1) Filter 1000 mL of sample water through 0.45 µm filter.
2) Add internal standard for loss recovery (benzo[a]pyrene) to sample water.
3) Condition the cartridge sequentially with:
    a. 4 mL ethyl acetate
    b. 4 mL dichloromethane
    c. 4 mL methanol
    d. 4 mL water
4) Load sample into conditioned cartridge
5) Air dry cartridge for 30 minutes.
6) Elute cartridge sequentially into marked glass vial with:
    a. 4 mL ethyl acetate
    b. 4 mL dichloromethane
7) Add (1:1) ethyl acetate/dichloromethane solution to glass vial until volume reaches 10 mL.
8) Air evaporate 4 mL of the solution.
9) Transfer into in clean GC vial.
10) Store indefinitely in 4 °C away from light.

Gas Chromatography Method

Accessories

1. Detector, flame ionization (FID)

2. Column, Rxi-17Sil, MS (15 m-long x 0.25 mm-internal diameter, 0.25 µm) (cat.# 14120)
3. Liner, 4 mm Split Precision Liner with glass wool (cat.# 21022)
4. Instrument, Shimadzu GC 2014

GC Operation Conditions (6440B-3c)

1. Sample
    1. Diluent: methylene chloride (care to avoid evaporation)
    2. Concentration: 20 ng/µL
2. Injection
    1. Volume: 1 µL split (split ration 20:1)
    2. Temperature: 275 °C
    3. Split vent flow rate: 42 mL/min
3. Oven
    1. Temperature: 80 °C (hold 1 min.) to 320 °C at 15 °C/min (hold 2 min.)
4. Carrier Gas
    1. Helium (He)
    2. Constant flow at 2 mL/min.
5. Detector
    1. Temperature: 340 °C
    2. Constant column + constant make-up: 50 mL/min.
    3. Gas type: Nitrogen (N_2)
    4. Data rate: 20 Hz

Quality Control/Quality Assessment (EPA Method 525.2-9.3, modified)

QA/QC performed at the beginning of each sample batch run and after every 20 samples

1. 4 replicas of analyte concentration in the middle of the calibration range.
    a. Add the appropriate aliquot of HCl and sodium sulfite to each analyte to standardize field and lab samples.
    b. For each analyte replica, the mean accuracy, expressed as a percentage of the true value should be between 70-130%.
    c. The relative standard deviation should be < 30%
2. Internal standard recovery should be > 70%
3. Laboratory fortified blank should be below the method detection limit
    a. Utilizing Method Detection Limit Calculator by the EPA

Water Characterization

Total Suspended Solids

Preliminary Work

1. Prepare the filters
    a. Hold the filters using cleaned forceps/tweezers.
    b. Drag the filter back and forth in ultrapure water ≈ 3 times, or until filter no longer gives off white residue.
    c. Place filters on a clean metal pan or aluminum sheet and dry in the oven at 120°C for 1 hour or until filter is completely dried.
    d. Immediately place filters in desiccator directly from the oven until cooled.

Sample Analysis

1. Place the cleaned filter on a piece of aluminum.
    a. Write the sample information on the aluminum with sharpie.
2. Weigh the clean filter and the aluminum and record the weight (W1)
3. Filter 100 mL of sample through the 0.45 μm filter.
    e. Handle the filter using only forceps/tweezers
4. Place filter on the corresponding aluminum foil and dry in oven at 103-105 °C for at least 1 hour.
5. Once dried, immediately place the filters in desiccator directly from the oven until cooled.
6. Weight the dried filter and aluminum foil (W2)
7. Follow the formula below for calculating TSS:

$$TSS\left({}^{g}/_{L}\right) = \frac{W2(g) - W1(g)}{mL\ of\ sample} * 1000$$

Conductivity

1. Submerge conductivity probe in sample
2. Wait until readout is stable
3. Record results in μS units

pH

1. Check probe accuracy
    a. Compare readout to standardized pH solutions on the counter (colorized)
    b. Calibrate if not within ± 0.25 units
2. Submerge pH probe in sample
3. Wait until readout is stable
4. Record results

Total Organic Carbon

Preliminary Work

1. Prepare 1 g-carbon/L stock organic carbon standards using potassium hydrogen phthalate (KHP)

    a. Weigh 1.0 g of KHP and dry in oven at 103-105 °C for 2-3 hours.
        i. Place immediately in desiccator directly from oven until cooled
    b. Weigh 0.53135 g of KHP and dissolve in ≈ 200 mL of ultrapure water.
    c. Add 6 N HCl until pH > 2 (check pH using pH strips by pipetting solution onto pH paper).
    d. Once pH is > 2, add the remaining ultrapure water until it reaches 250 mL.
    e. Store at 4 °C for no longer than 28 days.
2. Prepare working standard
    a. 20 mg/L, 25 mg/L, 125 mg/L

Sample Preparation/Analysis

1. Filter 30 mL of sample through 0.45 μm glass fiber filter.
    a. Make sure filter vacuum is thoroughly cleaned and dried, void of organic contaminants.
    b. If samples are turbid, homogenize the samples for 1 minute before filtering.
    c. If filtered sample is still visibility turbid, dilute it with ultrapure water (note the dilution factor).
2. Transfer filtered samples to ashed glass TOC vials and add small magnetic Teflon stir bar.
3. Run samples immediately.

QA/QC

1. Includes blank, ultrapure water, pass if < 0.5 mg/L
2. Matrix spike, spike concentration varies each run, pass if within 25% recovery
3. Standard, concentration varies each run, pass if within 10% of expected value.
4. Triplicates

Alkalinity (HACH/EPA Method 8203)

1. Select the sample volume and Sulfuric Acid (H_2SO_4) Titration Cartridge corresponding to the expected alkalinity concentration as mg/L calcium carbonate ($CaCO_3$) from Table 3.
2. Insert a clean delivery tube into the titration cartridge. Attach the cartridge to the titrator body.
3. Turn the delivery knob to eject a few drops of titrant. Reset the counter to zero and wipe the tip.
4. Use a graduated cylinder or pipet to measure the sample volume from Table 3. Transfer the sample into a clean 250-mL Erlenmeyer flask. Dilute to about the 100-mL mark with deionized water, if necessary.
5. Add the contents of one Phenolphthalein Indicator Powder Pillow and swirl to mix.
6. If the solution turns pink, titrate to a colorless end point. Place the delivery tube tip into the solution and swirl the flask while titrating with sulfuric acid. Record the number of digits required.
7. Calculate: Digits Required x Digit Multiplier = mg/L $CaCO_3$ Alkalinity.
8. Add the contents of one Bromcresol Green-Methyl Red Indicator Powder Pillow to the flask and swirl to mix.
9. Continue the titration with sulfuric acid to a light greenish blue-gray (pH 5.1), a light violet-gray (pH 4.8), or a light pink (pH 4.5) color, as required by the sample composition; see Table 4. Record the number of digits required.
10. Calculate: Total Digits Required x Digit Multiplier = mg/L as $CaCO_3$ Total Alkalinity

Table A3-3. Titration Cartridge corresponding to the expected alkalinity concentration as mg/L calcium carbonate ($CaCO_3$).

Range (mg/L as $CaCO_3$)	Sample Volume (mL)	Titration Cartridge (H_2SO_4)	Catalog Number	Digit Multiplier
10-40	100	0.1600	14388-01	0.1
40-160	25	0.1600	14388-01	0.4
100-400	100	1.600	14389-01	1.0
200-800	50	1.600	14389-01	2.0
500-2000	20	1.600	14389-01	5.0
1000-4000	10	1.600	14389-01	10.0

Table A3-4. Sample Composition and expected end point for alkalinity.

Sample Composition	End Point
Alkalinity about 30 mg/L	pH 4.9
Alkalinity about 150 mg/L	pH 4.6
Alkalinity about 500 mg/L	pH 4.3
Silicates or Phosphates present	pH 4.5
Industrial waste or complex system	pH 4.5

Methods resources

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Appendix 4: Data tables

Table A4-1: Wetlands microbial water quality

	Total coliforms		Fecal coliforms		Enterococci		Salmonella	
	MPN/100 mL		MPN/100 mL		MPN/100 mL		MPN/100 mL	
Sample	in	out	in	out	in	out	in	out
1 (wet)	1986.3	117.8	547.5	51.2	>2419.6	39.2	2.4	0.68
2 (wet)	>2419.6	17.3	547.4	28.3	>2419.6	19.5	1.175	8.75

Table A4-2: Wetlands inorganic constituents

	Cl^-		Br^-		SO_4^{2-}		I^-		NO_2^-		NO_3^-		PO_4^{3-}	
	mg/L		mg/L		mg/L		mg/L		mg/L as N		mg/L as N		mg/L	
Sample	in	out	in	out	in	out	in	out	in	out	in	out	in	out
1 (wet)	20.3	148	0	0	34.6	72.3	0	0	0.013 (BDL)	0.049	4.39	1.47	0.02	0.02
2 (wet)									0.036	0.349	4.58	0.719	0.06	0.04

Table A4-3: Wetlands general water quality

	TOC		BOD_5		TSS		Conductivity		pH		Alkalinity		Total Nitrogen		Total Phosphorus		COD	
	mg/L		mg/L		mg/L		µS				mg/L as $CaCO_3$		mg/L		mg/L		mg/L	
Sample	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	out
1 (wet)	8.6	7.1	5.6	3.7	293	12	616	921	6.90	6.97	143	204	8.33	2.67	4.24	1.97	27.0	31.0
2 (wet)	4.6	6.5	2.3	2.2	24	0.6	934	977	7.92	7.15	168	183	2.03	1.33	3.72	3.71	63.7	65.0

Table A4-4: AWPS microbial water quality

	Total coliforms		Fecal coliforms		Enterococci		Salmonella	
	MPN/100 mL		MPN/100 mL		MPN/100 mL		MPN/100 mL	
Sample	in	out	in	out	in	out	in	out
1 (wet)	>2419.6	0	>2419.6	0	>2419.6	0	8.75	<0.045
2 (wet)	>2419.6	0	>2419.6	0	>2419.6	0	27	<0.045

Table A4-5: AWPS inorganic constituents

	Cl^-		Br^-		SO_4^{2-}		I^-		NO_2^-		NO_3^-		PO_4^{3-}	
	mg/L		mg/L		mg/L		mg/L		mg/L as N		mg/L as N		mg/L	
Sample	in	out	in	out	in	out	in	out	in	out	in	out	in	out
1 (wet)	227	4.54	0.115	0.0242	216	1.62	0	0	0.22	0.00	10.1	1.02	0.02	0.01
2 (wet)									0.00	0.00	8.78	0.990	0.04	0.01

Table A4-6: AWPS general water quality

	TOC		BOD_5		TSS		Conductivity		pH		Alkalinity		Total Nitrogen		Total Phosphorus		COD	
	mg/L		mg/L		mg/L		µS				mg/L as $CaCO_3$		mg/L		mg/L		mg/L	
Sample	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	out	in	out
1 (wet)	7.6	0.1	7.0	0.3	4.5	0.8	1498	41.3	7.19	6.27	127	6.8	21.7	13.0	1.75	0.06	67	4.3
2 (wet)	10.9	0.1	11.9	BDL	7.4	0.2	1590	29.2	7.16	5.67	150	4.3	11.3	1.7	2.26	0.10	121	15

Table A4-7: Groundwater recharge monitoring well microbial water quality

Sample	Total coliforms	Fecal coliforms	Enterococci	Salmonella
	MPN/100 mL	MPN/100 mL	MPN/100 mL	MPN/100 mL
1 (wet)	0	0	0	<0.045
2 (wet)	0	0	0	<0.045

Table A4-8: Groundwater recharge monitoring well inorganic constituents

Sample	Cl^-	Br^-	SO_4^{2-}	I^-	NO_2^-	NO_3^-	PO_4^{3-}
	mg/L	mg/L	mg/L	mg/L	mg/L as N	mg/L as N	mg/L
1 (wet)	5.76	0.00	0	0	BDL	1.50	0.01
2 (wet)	5.54	0.07	1.91		0.018	1.48	0.01

Table A4-9: Groundwater recharge monitoring well general water quality

Sample	TOC	BOD_5	TSS	Conductivity	pH	Alkalinity	Total Nitrogen	Total Phosphorus	COD
	mg/L	mg/L	mg/L	µS		mg/L as $CaCO_3$	mg/L	mg/L	mg/L
1 (wet)	0.6	BDL	0.8	89.3	7.71	30.3	7.00	0.87	7.0
2 (wet)	0.8	1.0	0.1	99.2	7.06	29.8	1.67	0.63	4.3

Table A4-10: Lake microbial water quality

	Total coliforms			Fecal coliforms			Enterococci			Salmonella		
	MPN/100 mL			MPN/100 mL			MPN/100 mL			MPN/100 mL		
Sample	WW	Mix	DW	WW	Mix	DW	WW	Mix	DW	WW	Mix	DW
1 (dry)	1	1	3	1	0	3.1	0	0	0	0.23	0.1	0.65
2 (dry)	13.4	2	1	9.8	0	0	0	0	0	0.1	0.1	0.225
3 (wet)	9.6	14.8	129.1	3.1	9.6	82	0	0	20.3	0.09	1.3	46

Table A4-11: Lake inorganic constituents

	NO_2^-			NO_3^-			PO_4^{3-}		
	mg/L as N			mg/L as N			mg/L		
Sample	WW	Mix	DW	WW	Mix	DW	WW	Mix	DW
1 (dry)	BDL	BDL	BDL	4.15	0.226	0.241	0.01	0.20	0.01
2 (dry)	BDL	BDL	BDL	4.64	0.273	0.265	0.02	0.02	0.02

Table A4-12: Lake general water quality

	TOC			BOD_5			TSS			Conductivity		
	mg/L			mg/L			mg/L			µS		
Sample	WW	Mix	DW	WW	Mix	DW	WW	Mix	DW	WW	Mix	DW
1 (dry)	7.2	2.0	1.8	1.0	0.4	0.4	0.6	1.4	1.0	502	61.9	59.2
2 (dry)	8.3	1.7	1.6	1.6		1.0	1.6	2.6	1.2	483	65.1	65.4
3 (wet)	7.1	1.8	2.2									

	pH			Alkalinity			Total Nitrogen			Total Phosphorus			COD		
				mg/L as $CaCO_3$			mg/L			mg/L			mg/L		
Sample	WW	Mix	DW	WW	Mix	DW	WW	Mix	DW	WW	Mix	DW	WW	Mix	DW
1 (dry)	7.61	7.22	6.83	121	12.3	13.0	5.00	2.33	0.00	0.87	0.18	0.11	46	15	12
2 (dry)	7.06	7.16	6.80	107	13.7	13.0	3.33	4.33	0.67	0.45	0.27	0.14	52	11	14

Field testing of mesocosm-scale derived nitrate removal models to verify water quality improvement potential of restored coastal forested wetlands

Basic Information

Title:	Field testing of mesocosm-scale derived nitrate removal models to verify water quality improvement potential of restored coastal forested wetlands
Project Number:	2016NC209B
Start Date:	9/1/2016
End Date:	8/31/2017
Funding Source:	104B
Congressional District:	NC-04
Research Category:	Water Quality
Focus Category:	Agriculture, Wetlands, None
Descriptors:	None
Principal Investigators:	Michael Burchell, Jalmar Kurki-Fox

Publications

There are no publications.

Field testing of mesocosm-scale derived nitrate removal models to verify water quality improvement potential of restored coastal forested wetlands

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WRRI Project # 16-13-W

May 15, 2017

1. SUMMARY

1.1 Activities and Findings

The project has encountered significant delays due the landowner's inaction on installing a new water control structure (WCS) at the restored wetland site and cleaning out other WCSs downstream from the site. To date, no pumping and drawdown trials have been completed. However, the WCS has been delivered in the past week, and the site should be ready for testing by the end of May. Activates completed to date include:

- The WRRI/Sea Grant funding has been used to leverage an additional $7,700 in funding from the Coastal Federation to enhance hydrologic monitoring and assess whether the restored wetland functions as a source or sink of bacteria. Fecal coliform bacteria is a significant concern for shellfishing in the coastal waters of NC.
- Equipment has been purchased and is ready for installation once the WCS is installed at the wetland cell.
- Monitoring plan finalized.

1.2 Deviations from Original Project Plans

The project site has been moved to a different restored wetland cell about two miles away from the original site. The new restored wetland cell is smaller than the previous site (50 ac. Vs. 300 ac.), which will allow for more accurate quantification of the results. The original cell's topography would have led to significant short-circuiting, and unforeseen hydraulic constraints downstream of the restored cell may have prevented the cell from draining adequately. The project has been expanded with additional funding to include monitoring bacteria in the wetland cell and surrounding waters.

2. REFERENCES

N/A

3. APPENDIX 1

N/A

4. APPENDIX 2

N/A

Understanding how land use characteristics affect the prevalence of antibiotic resistant, virulent E. coli and host-specific markers in watersheds with and without swine CAFOs

Basic Information

Title:	Understanding how land use characteristics affect the prevalence of antibiotic resistant, virulent E. coli and host-specific markers in watersheds with and without swine CAFOs
Project Number:	2016NC210B
Start Date:	9/1/2016
End Date:	8/31/2017
Funding Source:	104B
Congressional District:	NC-04
Research Category:	Biological Sciences
Focus Category:	Water Quality, None, None
Descriptors:	None
Principal Investigators:	Jill Stewart, Elizabeth Christenson

Publications

There are no publications.

Mid-year report for
North Carolina Sea Grant and Water Resources Research Institute

Submitted April, 21, 2017

Reporting for September 1, 2016 through January 31, 2017

Title: **Understanding how land use characteristics affect the prevalence of antibiotic resistant, virulent *E. coli* and host-specific markers in watersheds with and without swine CAFOs**

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Original objectives:

This project will systematically compare watersheds with different land use characteristics, primarily with respect to swine concentrated animal feeding operations (CAFOs), and their effect on microbial water quality. The hypothesis is that **antibiotic resistant *E. coli*, virulent *E. coli*, and swine-specific MST markers will be higher in watersheds with swine CAFOs compared to similarly sized agricultural watersheds without any CAFOs.** The specific research objectives of this project are to (1) quantify microbial pollution as defined by *E. coli*, (2) quantify virulent *E. coli*, (3) quantify microbial source tracking (MST) indicators to identify sources of *E. coli*, (4) determine antibiotic resistance of *E. coli*, and (5) use exploratory research techniques to assess potential relationships of spatial covariates with microbial outcomes between ten watersheds with swine CAFOs and ten similarly sized and used watersheds without swine CAFOs in NC.

Research progress:

Sample sites:

Background sites are defined as watershed land area upstream of sampling point that are primarily agricultural land not containing any type of CAFO, wastewater treatment plant. Swine sites are defined as watershed land area upstream of sampling point that are primarily agricultural land containing a swine CAFO barn and/or lagoon and/or sprayfield and does not have any other kind of CAFO or wastewater treatment plant. Nine background sites and thirteen swine sites have been sampled four times between September 1, 2016 and January 31, 2017. This corresponds to 44% of planned nine sampling events. Approximately one liter of water was collected at each sampling site and sampling time.

E. coli culture and quantification:
For all collected water samples, membrane filtration was conducted. 44% of planned membrane filtration and *E. coli* culture and quantification has been completed. Average concentration *E. coli* in background sites was 145 colony forming units (CFU)/100mL compared to average concentration of 379 CFU/100mL in swine sites. 422 *E. coli* isolates confirmed as indole producers have been archived.

qPCR and microbial source tracking:
Microbial source tracking has not yet been conducted.

Virulence testing
Virulence testing has not yet been conducted.

Antimicrobial resistance testing:
Of 422 *E. coli* isolates archived, 68 background and 116 swine *E. coli* isolates have been tested for resistance to eleven antibiotics: Amoxicillin-clauvanate acid (AmC), ampicillin (AM), cefoxitin (FOX), ceftriaxone (CRO), chloramphenicol (C), ciprofloxacin (CIP), imipenem (IMP), gentamycin (GM), levofloxacin (LVX), tetracycline (TE), and sulfamethoxazole-trimethoprim (SXT).

Table 1 displays the results of antimicrobial resistance testing between September 2016 and January 2017.

Table 1: Number and percent of *E. coli* isolates with observed resistance to antibiotics from water samples collected from background and swine sites. Observed resistance does not include observed intermediate resistance.

	AmC	**AM**	**FOX**	**CRO**	**C**	**CIP**	**GM**	**LVX**	**TE**	**SXT**	**IMP**
Background	0	0	0	0	0	0	0	0	4 (6%)	0	0
Swine	1 (1%)	9 (8%)	1 (1%)	2 (2%)	1 (1%)	0	0	0	33 (29%)	0	0

Multi-drug resistance defined as resistance to three or more antibiotic classes was observed for three swine isolates.

Resistance has not been observed in any sample to antibiotics imipenem, ciprofloxacin, gentamycin, levofloxacin, and sulfamethoxazole-trimethoprim.

Analysis planned:

Sample collection, *E. coli* culture and quantification, and antimicrobial resistance testing will continue through August 2017. Antimicrobial resistance testing will additionally incorporate a confirmation test of ESBL and Amp-C producing *E. coli.* Microbial source tracking will be conducted summer 2017 using droplet digital PCR (ddPCR) to target pig-specific fecal marker pig-2-bac and human-specific fecal marker HF183. Virulence testing will be conducted summer 2017 on archived *E. coli* isolates collected at NCSU with Dr. Megan Jacob.

Analysis will incorporate spatial variables, and prior precipitation.

Training:

This fellowship provided funding for one PhD student and one undergraduate student to attend the annual WRRI conference in Raleigh, NC in March 2017.

Additionally, implementation of this project has included training of five undergraduates to help with laboratory work including media preparation, antibiotic resistance testing, membrane filtration, and *E. coli* culture and isolation.

Oral presentations:

Christenson, E., Stewart, J. Prevalence of antibiotic-resistant *E. coli* in North Carolina watersheds with and without swine CAFOs. Water Resources Research Institute annual conference. Raleigh, NC. March 16, 2017.

Christenson, E. Stewart, J. All that glimmers is not gold: Understanding how land use characteristics affect the prevalence of antibiotic resistant *E. coli* in watersheds with and without swine CAFOs. Environmental Sciences and Engineering department seminar. Chapel Hill, NC. March 1, 2017.

Information Transfer Program Introduction

The Water Resources Research Institute (WRRI) is designed to provide water resources information to a range of stakeholders including private industry, academics, non-profit groups, and governmental entities. WRRI maintains a strong information transfer program by cooperating with various state agencies, municipalities, and professional organizations to sponsor conferences, workshops and other educational events, as well as seeking grants for relevant activities and publishing and distributing research results.

WRRI continues to administer the NC Urban Water Consortium (UWC) and the UWC-Stormwater Group (SWG), which comprise drinking/wastewater utilities and municipal stormwater programs, respectively. WRRI plays an active role in developing agendas for quarterly meetings for each group (a total of 8 held during this reporting period) that highlight emerging priority research projects in the state, exploring topics of concern for each group, and pursuing opportunities to educate and engage group members to better enable their management activities.

WRRI continues to sponsor continuing education credits by the NC Board of Examiners of Engineers and Surveyors as an Approved Sponsor of Continuing Professional Competency activity for Professional Engineers and Surveyors licensed by the State of North Carolina. In addition, WRRI submits information for approval to the N.C. Board of Landscape Architects to offer contact hours to landscape architects. This allows WRRI to offer Professional Development Hours (PDHs) to engineers and surveyors, and Continuing Education Units (CEUs) to landscape architects for attendance at the WRRI Annual Conference and other workshops, seminars and forums that WRRI sponsors.

WRRI continues to expand its activities under the umbrella of the Center of Excellence for Watershed Management (CEWM). Through the CEWM, WRRI's Sustainable Waters and Communities Coordinator helps communities identify local opportunities and implement sustainable practices for managing their waters. Community leadership and participation in watershed efforts are paramount to protecting waters, and the CEWM provides services and support for these efforts. The CEWM aids communities by supporting the NC Watershed Stewardship Network (NCWSN), providing tools and training opportunities, and coordinating local watershed specific projects. The NCWSN continues to grow in size, scope and network-sponsored activities. The NCWSN is guided by a Steering Committee of twenty four people from watershed organizations across the state, and is coordinated in partnership with the UNC Institute for the Environment.

WRRI Information Transfer Program

Basic Information

Title:	WRRI Information Transfer Program
Project Number:	2016NC204B
Start Date:	3/1/2016
End Date:	2/28/2017
Funding Source:	104B
Congressional District:	NC-004
Research Category:	Not Applicable
Focus Category:	None, None, None
Descriptors:	None
Principal Investigators:	Nicole Wilkinson

Publications

There are no publications.

FY 2016 Information Transfer Program Progress & Achievements

WRRI-SPONSORED WORKSHOPS, FORUMS AND SEMINARS

Below is a list of the educational and training events WRRI sponsored during the project year, along with a description of each and the number of attendees. Through these events and programs, WRRI engaged a documented 854 participants, though many events targeted additional unquantified audiences through webinars and public gatherings where participant numbers were not known.

WRRI 18th Annual Conference and NCWRA Symposium

For 18 years, the WRRI Annual Conference has been the premier conference highlighting diverse topics in water research, management and policy in North Carolina. The conference featured oral and poster presentations, themed panel discussions, ample networking opportunities, and hands-on interactive sessions for more in-depth discussions and problem solving related to water resources. The conference was held in conjunction with the NC Water Resources Association's Annual Symposium, "Blue to Green: The Economic Value of Water." Water is NC's greatest asset and greatest amenity, supporting people, agriculture, industry, ecosystems, recreation, a burgeoning brewery scene, and much more. The symposium will explore the many ways we value water and demonstrate the fundamental role that water plays in our vibrant economy. 261 people participated on March 17-18, 2016.

Erosion and Sedimentation Control Planning and Design Workshop

This workshop was structured to educate and familiarize design professionals with the NC Sedimentation Pollution Control Act (SPCA), the rules implementing the Act, design standards for erosion and sedimentation control BMPs and elements that are necessary to submit an erosion control plan. This workshop focused on legislative updates in NC, ESC case study, updates on Stormwater MDC Program, recent technological advances in ESC, overview of E-permitting systems used by various local governments and ESC forestry operations used in NC. 66 people participated in this event on April 28, 2016.

Tools of Watershed Management Workshop Series

Forty two local watershed stewards participated in three 2-day workshops held in Salisbury, Asheville, and Kinston, NC to learn about watershed planning. Led by partners WRRI and UNC Institute for the Environment and presented by members of the NC Watershed Stewardship Network, the workshops brought in local guest watershed speakers and engaged participants from the regions in exercises to learn about planning for watershed restoration and protection. A participant at the Kinston workshop summarized their thoughts about the workshop by saying, "This was an extremely informative experience and great opportunity to meet other people working on watershed in our area."

Multiple Dates:
April 12-13, 2016 - Salisbury
May 17-18, 2016 - Asheville

June 15-16, 2016 – Kinston

WRRI Research Funding Leads To Development Of Integrated Water Management Portal

The WRRI Urban Water Consortium, a research collaborative of NC's twelve largest drinking and wastewater utilities, along with WRRI core funds have supported research from 2011-2015 for the development of an integrated water management portal. Funding supported NCSU researcher Sankar Arumugam, his graduate student Amir Mazrooei, and the State Climate Office of NC to incorporate climate models, forecasts and observed conditions into a single portal that water utilities and the Army Corps of Engineers can use to predict streamflow and drinking water reservoir levels. This information can lead to more reliable management decisions about retaining or releasing water, changing operations to encourage water conservation at the utility and among citizens if needed, and balancing upstream and downstream needs. WRRI worked closely with the research team to host a webinar on May 13, 2016, in which 21 water resource management professionals from local utilities, state government, academia and private consulting participated. A follow up, in-person training was held on June 29, 2016 to provide users with a more in-depth hands-on demonstration and exercises to better enable users to apply the portal tool to their work. This years-long effort highlights an ideal progression from an initial research concept (utilizing climate models to predict streamflow forecasts), to the development of a management tool that is specific to North Carolina and unique in the nation, to the training and application of the tool by decision makers.

Water Resources Advisory Committee and Smart Growth Committee: Green Infrastructure in Private Development

The Triangle J Council of Governments Water Resources Advisory Committee and Smart Growth Committee held a joint meeting on a topic of shared interest, and was sponsored by NC WRRI. Last year they focused on local government implementation of green infrastructure projects. There has been continuing interest in this topic, so this year they looked at green infrastructure from a private development standpoint, with a specific focus on water. Well-known speakers discussed how they've incorporated green infrastructure into development projects in the Triangle. The agenda included: One Water - a paradigm for the future; a renewed way of thinking about water by Trevor Clements of Tetra Tech Inc. Chatham Park - designing green infrastructure at scale by Charles R. (Chuck) Smith, PLA, ASLA, of Preston Development Company and a look at Market at Colonnade in North Raleigh - The data is in, how well has the green infrastructure performed? by William F. (Bill) Hunt, III, PhD, P.E., D.WRE, of NC State University. 31 people participated in this event in Durham, NC on July 28, 2016.

NCWRA Forum: Leading (and Following) During Adversity: Lessons from Coal Ash

The coal ash accident at Duke Energy's Dan River Steam Station in 2014 fundamentally altered the way many think about electricity. Mark McIntire of Duke Energy shared his coal ash story and how the events of February 2014 are changing an industry. In addition

to answering questions about the accident and all that has transpired since, he also shared his perspectives on environmental leadership during difficult times. 89 people participated in this event on September 12, 2016.

Duke Energy Erosion & Sedimentation Control Workshop

This year, WRRI formed a new working relationship with industry partner Duke Energy. Duke Energy representatives approached WRRI in spring 2016 with interest in developing an erosion and sedimentation control workshop explicitly for Duke Energy engineers, inspectors and consultants. Recognized for our ability to coordinate this type of training, WRRI has supported erosion and sedimentation control educational efforts in North Carolina for decades in various capacities. The day-long workshop, held on November 7, 2016, at the Jane S. McKimmon Center in Raleigh, NC offered 6.5 Professional Development Hours to 170 in-person attendees with several other groups around the state participating virtually in the event via webinar. Multiple groups within Duke Energy also viewed the recorded webinar on two other dates after Nov 7. WRRI expects to continue this partnership with Duke Energy and provide similar events in 2017. Using feedback and evaluation results from the initial workshop, we intend to pare down audience size and tailor the technical information presented to specific subsets of Duke Energy employees.

NCWRA Forum: Green Infrastructure – The Future of Stormwater

In response to massive fish kills in the 1990's and other on-going water quality concerns, North Carolina has focused on innovative stormwater management for two decades. Many treatment techniques have been utilized in the state, many of which are based upon research conducted at NC State University. This presentation discussed evolution practices used across North Carolina and the various concerns raised by the design community (i.e. maintenance, reliable tools, and needed metrics). Highlighted practices included bioretention, permeable pavement, constructed wetlands, and wet pond retrofits. 72 people participated in this event on December 5, 2016.

NCWRA Forum: What's in Wastewater: Bacteria, Viruses & Parasite Pathogens, Plus Their Genes: What we Know and Need to Know Better.

Human and animal wastewater contains high concentrations of bacteria, viruses and parasites, which can cause waterborne diseases in humans via exposure through drinking and recreational waters. Given the diversity of potential pathogens, we rely on fecal indicators to inform us about their presence and potential risks to human health. Historically, fecal indicators were only used to detect and quantify bacteria but similar methods are now being developed to test for viruses and protozoan parasites. Greater use of these additional fecal indicators should be encouraged to better manage human health risks from these pathogens and may be required by future water quality criteria and standards. Dr. Mark Sobsey of UNC-Chapel Hill addressed these topics in his presentation. 59 people participated in this event on February 6, 2017.

CENTER OF EXCELLENCE FOR WATERSHED MANAGEMENT

NC Watershed Stewardship Network

In FY16, WRRI continued its commitment to the NC Watershed Stewardship Network (WSN), which was formed through a collaboration in which WRRI was highly active and engaged, with continued funding for its Sustainable Waters and Communities Coordinator to serve part-time as co-coordinator of the network. During this time period, the WSN continued to connect watershed stakeholders from around the state with each other as well as provide access to local watershed data, and continued to host steering committee meetings at regular intervals around the state. The WSN co-sponsored a watershed workshop series described above.

Community watershed restoration efforts

The Sustainable Waters and Communities Coordinator continues to manage two community watershed restoration efforts funded and supplemented by EPA 319 grants and cost-sharing contributed by partnering organizations. These include the Black Creek Watershed Association in the Neuse River Basin and the town of Cary; the Burnt Mill Creek Watershed Initiative in the Cape Fear River Basin and the city of Wilmington; and the Walnut Creek Wetland Community Partnership in southeast Raleigh. These projects involve engaging local municipal and citizen partners in education, installing stormwater control measures to reduce urban runoff, and monitoring impacts. In FY 16, the CEWM engaged 64 K-12 students in community projects to protect and restore watersheds, including:

- 55 Kingswood Elementary School 5th graders engaged in an interactive guest session about benthic macro-invertebrates and what they tell us about stream health in November 2016. This effort was upported by US EPA 319 funds.
- 5 teens participated in an informal “Big Sweep” event for Black Creek in April 2016.
- 4 teens from Green Hope High School volunteered for rain garden maintenance event in September 2016.

The Sustainable Waters and Communities Coordinator also continued efforts with the DREAMS Youth Program. This project engaged an after-school arts organization for at-risk youth in the City of Wilmington in the sustainable rehabilitation of a degraded parking lot. DREAMS of Wilmington is located within the degraded Burnt Mill Creek watershed, the subject of an ongoing watershed restoration effort. To reduce stormwater running off of the site, the parking lot was deconstructed and rebuilt with a large bioretention area and permeable parking stalls. A project goal has been to foster a strong connection between DREAMS’ students and their immediate environment, in particular increasing their understanding of stormwater and associated environmental issues, and deepening their interest in and concern for our natural world. This was accomplished by engaging teaching staff and students at points throughout the brainstorming, design, and installation of the project. Students were engaged in educational hands-on activities about watershed science, and went on field trips to see how water moves across the landscape from DREAMS down to the coast. The project was awarded an Outstanding Stewardship Award by the Lower Cape Fear Stewardship

Development Association in February, 2017. WRRI was the lead, and partners included City of Wilmington and NC State University Department of Biological and Agricultural Engineering, with funding provided by the US EPA Clean Water Act Section 319.

PUBLICATIONS

Seven Peer-Reviewed Publications Resulted from WRRI-Funded Projects:

- Sun, M., Lopez-Velandia, C., and Knappe, D.R.U. 2016. "Determination of 1,4-dioxane in the Cape Fear River watershed by heated purge-and-trap preconcentration and gas chromatography–mass spectrometry." Environmental Science and Technology, 50 (5), pp 2246–2254.
- Shifflett, SD; Culbreth, A; Hazel D; Daniels, H; Nichols, EG. 2016 Coupling aquaculture with forest plantations for food, energy, and water resiliency. Sci Total Environ (2016), http://dx.doi.org/10.1016/j.scitotenv.2016.07.161
- Brandt, J.E.; Bernhardt, E.S.; Dwyer, G.S.; and Di Giulio, R.T.; 2017. Selenium ecotoxicology in freshwater lakes receiving coal combustion residual effluents: A North Carolina example. Environ Sci & Technol. Article ASAP DOI: 10.1021/acs.est.6b05353.
- Lopez, A.R., D.H. Funk and D.B. Buchwalter. 2017. Arsenic (V) bioconcentration kinetics in freshwater macroinvertebrates and periphyton is influenced by pH. Environmental Pollution. 224:82-88.
- Lopez, A.R., D.R. Hesterberg, D.H. Funk, and D.B. Buchwalter. 2016. Bioaccumulation dynamics of arsenate at the base of aquatic food webs. Environmental Science and Technology. 50: 6556-6564.
- Vengosh, A. et al. 2016. Origin of Hexavalent Chromium in Drinking Water Wells from the Piedmont Aquifers of North Carolina. Environ. Sci. Technol. Lett. 3, 409-414
- Cheng, Q.; Call, D. F. Hardwiring microbes via direct interspecies electron transfer: mechanisms and applications. Environ. Sci. Process. Impacts 2016, 18(8), 968–980

WRRI published 13 (thirteen) internal research reports during this reporting period for projects that were finalized during this period:

- Report UNC-WRRI-466 by Michael Mallin "Quantification of Fecal Bacteria Removal by Microzooplankton Grazing in Stormwater BMPs" available at go.ncsu.edu/14-02-W
- Report UNC-WRRI-477 by Alex Manda "Coastal Groundwater Watch: A Citizen Science Project" available at go.ncsu.edu/14-09-WSG
- Report UNC-WRRI-478 by Detlef Knappe "Occurrence of 1,4-Dioxane in the Cape Fear River Watershed and Effectiveness of Water Treatment Options for 1,4-Dioxane Control" available at go.ncsu.edu/14-06-U
- Report UNC-WRRI-479 by Alexandra Hounshell "Role of Organic Nitrogen to Eutrophication Dynamics Along the Neuse River Estuary, NC" available at go.ncsu.edu/16-06-W
- Report UNC-WRRI-480 by Tiffany Messer "Predicting Water Quality Impacts of Rerouting Drainage Water from the Pamlico Sound to Restored Wetlands" available at go.ncsu.edu/13-03-W

- Report UNC-WRRI-481 by Michael Vepraskas "Phosphorus Fluxes in a Restored Carolina Bay Wetland Following Eight Years of Restoration" available at go.ncsu.edu/12-02-W
- Report UNC-WRRI-460 by Martin Tsui "Linkages of mercury and methane cycles in Piedmont streams and rivers in North Carolina, and implications for mercury bioaccumulation in food webs" available at go.ncsu.edu/14-04-W
- Report UNC-WRRI-461 by Harry Daniels "Land Application of Aquaculture Effluents to Prevent Surface Water Eutrophication and Promote Groundwater Re-Infiltration in Coastal North Carolina" available at go.ncsu.edu/14-07-WSG
- Report UNC-WRRI-462 by William Hunt "Nutrient and Carbon Loading in Gross Solids in Urban Catch Basins: A Nutrient Accounting Opportunity?" available at go.ncsu.edu/13-09-S
- Report UNC-WRRI-463 by Anne Hershey "Heavy metal analysis, gene proxies, and stable isotopes tracers of coal ash contamination in the Dan River food web" available at go.ncsu.edu/15-02-W
- Report UNC-WRRI-464 by Richard DiGiulio "Legacy impacts of coal combustion residues on freshwater ecosystems in North Carolina" available at go.ncsu.edu/15-03-W
- Report UNC-WRRI-465 by David Buchwalter "Coal ash constituents at the base of aquatic food webs: Processes affecting bioaccumulation and trophic transfer of arsenic" available at go.ncsu.edu/15-04-W

ONLINE RESOURCES

WRRI overhauled its website and launched a new version, wrri.ncsu.edu, in August 2015, and the new site continued to be an effective medium for communication and information sharing in FY16. This revision brought WRRI's site into alignment with NC State University's branding efforts, reflects current trends in website appearance and functionality, and is a great improvement in how WRRI showcases its impacts and achievements. WRRI also continues to grow its online presence through the use of a twitter account (@NC_WRRI), through which it shares WRRI-generated research results, news items, and other relevant water-related information.

WRRI ELECTRONIC LISTS

WRRI maintains the following electronic mail lists (listservs) for information transfer purposes, which reach a combined total of almost 2000 people statewide:

- Water-Research list -– informs water researchers from NC universities about calls for papers, grants, upcoming conferences, student internships, etc.;
- WRRI-News list - informs researchers, local governments, municipalities, interest groups etc. about calls for papers, grants, upcoming conferences and events, etc.;
- NCWRA-info list - provides information of the North Carolina Water Resources Association sponsored events;
- Sediments list - used to disseminate erosion and sedimentation control information in North Carolina;
- Watershed Stewardship Network (WSN) list – provides watershed professionals, volunteers and stakeholders throughout the state with a mechanism to contact,

network, and learn from each other as well as to learn about the WSN and its offerings;
- Urban Water Consortium (UWC) list for Urban Water Consortium member communications;
- and UWC-Stormwater Group list for the UWC Stormwater Group member communications.

NC URBAN WATER CONSORTIUM

WRRI administers the NC Urban Water Consortium (UWC) and meets with the members quarterly. The consortium was established in 1985 by the Institute, in cooperation with several of North Carolina's larger cities to provide a program of research and development, and technology transfer on water problems that urban areas share. Through this partnership, WRRI and the State of North Carolina help individual facilities and regions solve problems related to local environmental or regulatory circumstances. Participants support the program through annual dues and enhancement funds and guide the program through representation on an advisory board, selection of research topics, participation in design of requests for proposals, and review of proposals. There are 12 member cities/special districts in North Carolina, and members hosted four quarterly meetings throughout the state in FY16.

NC URBAN WATER CONSORTIUM - STORMWATER GROUP

In 1998, several members of the NC UWC partnership formed a special group to sponsor research and technology transfer on issues related to urban stormwater and management. The Urban Water Consortium (UWC) Stormwater Group is administered by WRRI. Participants support the program through annual dues and enhancement funds. They guide the program through selective representation on the WRRI advisory board, determining stormwater-related research priorities, participation in the design of requests for proposals and review of proposals submitted to WRRI directly or to the SWG. Four meetings were hosted by rotating SWG members throughout the state during the reporting year.

USGS Summer Intern Program

None.

Student Support					
Category	**Section 104 Base Grant**	**Section 104 NCGP Award**	**NIWR-USGS Internship**	**Supplemental Awards**	**Total**
Undergraduate	5	1	0	6	12
Masters	4	0	0	2	6
Ph.D.	7	1	0	7	15
Post-Doc.	0	0	0	0	0
Total	16	2	0	15	33

Notable Awards and Achievements

SUSTAINABLE PARKING LOT RECEIVES LOWER CAPE FEAR STEWARDSHIP DEVELOPMENT AWARD This project engaged an after-school arts organization for at-risk youth in the City of Wilmington in the sustainable rehabilitation of a degraded parking lot. DREAMS of Wilmington is located within the degraded Burnt Mill Creek watershed, the subject of an ongoing watershed restoration effort. To reduce stormwater running off of the site, the parking lot was deconstructed and rebuilt with a large bioretention area and permeable parking stalls. A project goal has been to foster a strong connection between DREAMS' students and their immediate environment, in particular increasing their understanding of stormwater and associated environmental issues, and deepening their interest in and concern for our natural world. This was accomplished by engaging teaching staff and students at points throughout the brainstorming, design, and installation of the project. Students were engaged in educational hands-on activities about watershed science, and went on field trips to see how water moves across the landscape from DREAMS down to the coast. The project was awarded an Outstanding Stewardship Award by the Lower Cape Fear Stewardship Development Association in February, 2017. WRRI was the lead and partners included City of Wilmington and NC State University Department of Biological and Agricultural Engineering, with funding provided by the US EPA Clean Water Act Section 319.

TOOLS OF WATERSHED MANAGEMENT WORKSHOP HELPS LOCAL STEWARDS' PREPARE FOR PLANNING In late spring to early summer of 2016, forty two local watershed stewards participated in workshops held in Salisbury, Asheville, and Kinston, NC to learn about watershed planning. Led by partners WRRI and UNC Institute for the Environment and presented by members of the NC Watershed Stewardship Network, the workshops brought in local guest watershed speakers and engaged participants from the regions in exercises to learn about planning for watershed restoration and protection. A participant at the Kinston workshop summarized their thoughts about the workshop by saying, "This was an extremely informative experience and great opportunity to meet other people working on watershed in our area."

STUDENT AWARDS AND ACHIEVEMENTS Noyes Harrigan received two awards for his poster presentations that were based upon the work from the project "Comparing the impact of organic vs. inorganic nitrogen loading to the Neuse Estuary with a mechanistic eutrophication model", lead by PI James Bowen of UNC-Charlotte and funded by the NC WRRI. The first award was received at the Water Smart Innovations Conference in Las Vegas, NV from October 5-7, 2016. He attended the conference as the winner of a WRRI travel scholarship. His poster "Three for the Price of Two? A comparison of circulation in the Neuse River Estuary predicted by a two and three-dimensional model" won the best student poster competition at the conference. The poster was also presented at the NC American Water Works Association (AWWA) Conference where it also won the best student poster competition. The poster was also presented at the NC WRRI Annual Conference March 15-16, 2017. Mr. Harrigan also received a teaching assistantship from the Department of Civil and Environmental Engineering at UNC Charlotte as a result of the WRRI funding award.

WRRI-SPONSORED RESEARCH LEADS TO ADDITIONAL FUNDING AWARDS Ms. Alex Hounshell was a recipient of one of the inaugural WRRI student research awards for her project "Role of organic nitrogen to eutrophication dynamics along the Neuse River Estuary, NC", which she worked on under the advising of Dr. Hans W. Paerl of UNC-Chapel Hill and Dr. Christopher L. Osburn of NC State University. Research conducted as part of the WRRI award was used as a foundation for a NSF RAPID Collaborative Research grant "Carbon and nutrient responses in an estuarine-coastal complex impacted by floodwaters from Hurricane Matthew", awarded to Drs. Paerl and Osburn in the amount of $169,763. Results from Ms. Hounshell's project will be used as the baseline for which results from the NSF RAPID project are compared to.

Mr. Bryan Maxwell, another of the inaugural student RFP award recipients, received supplemental funding for his WRRI-funded project “Quantifying treatment potential of floating treatment wetlands to benefit North Carolina waters using improved methodology and novel technology” from the NC State University Sustainability Fund in the amount of $12,630. He was also awarded a $2500 ACCIAC Research Fellowship for “Microbial Community Analysis within Stormwater Floating Islands in North Carolina.”

Dr. Doug Call’s PhD student, Qiwen Cheng, received an award from P.E.O. International Peace Scholarship Fund in the amount of $12,500. Her application included many lessons learned from her research funded by WRRI for the project “Improving the Anaerobic Treatment of Sludges and High-Strength Wastewaters through Addition of Electrically-Conductive Particles”. The funds, received in academic year 2017-2018, will be used to support her graduate studies in the same topical area.

SERVICE ON BOARDS AND COMMITTEES WRRI team members are actively engaged in board and committee activities around the state where they bring expertise and perspective to efforts to address NC’s water issues. WRRI is represented on the following: - NC Water Resources Association Board of Directors - NC Sedimentation Control Commission - NC Nutrient Criteria Implementation Committee - NC Defense Coastal/Estuarine Research Program Regional Coordinating Committee - Greater Triangle Stewardship Development Association Board of Directors - Universities Council on Water Resources (UCOWR) Board of Directors

Publications from Prior Years

1. 2015NC193B ("Coal ash constituents at the base of aquatic food webs: Processes affecting bioaccumulation and trophic transfer of arsenic") - Articles in Refereed Scientific Journals - Lopez, A.R., D.H. Funk and D.B. Buchwalter. 2017. Arsenic (V) bioconcentration kinetics in freshwater macroinvertebrates and periphyton is influenced by pH. Environmental Pollution. 224:82-88.
2. 2015NC193B ("Coal ash constituents at the base of aquatic food webs: Processes affecting bioaccumulation and trophic transfer of arsenic") - Articles in Refereed Scientific Journals - Lopez, A.R., D.H. Funk and D.B. Buchwalter. 2017. Arsenic (V) bioconcentration kinetics in freshwater macroinvertebrates and periphyton is influenced by pH. Environmental Pollution. 224:82-88.
3. 2015NC192B ("Legacy impacts of coal combustion residues on freshwater ecosystems in North Carolina") - Articles in Refereed Scientific Journals - Brandt, J.E.; Bernhardt, E.S.; Dwyer, G.S.; and Di Giulio, R.T.; 2017. Selenium ecotoxicology in freshwater lakes receiving coal combustion residual effluents: A North Carolina example. Environ Sci & Technol. Article ASAP DOI: 10.1021/acs.est.6b05353.
4. 2014NC190B ("Land Application of Aquaculture Effluents to Prevent Surface Water Eutrophication and Promote Groundwater Re-Infiltration in Coastal North Carolina") - Articles in Refereed Scientific Journals - Shifflett, SD; Culbreth, A; Hazel D; Daniels, H; Nichols, EG. 2016 Coupling aquaculture with forest plantations for food, energy, and water resiliency. Sci Total Environ (2016), http://dx.doi.org/10.1016/j.scitotenv.2016.07.161